FINAL REPORT OF THE LUANGWA SLEEPING SICKNESS COMMISSION OF THE BRITISH SOUTH AFRICA COMPANY

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AND

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ENTOMOLOGIST TO THE COMMISSION

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INTRODUCTION

During the years 1909 and 1910, the diagnosis of several cases of human trypanosomiasis amongst Europeans, who had never been in contact with *Glossina palpalis*, drew attention to the occurrence of the infection in portions of Rhodesia and Nyasaland in which this particular insect was not known to exist. As the result of prolonged and careful search, it was definitely proved that *Glossina palpalis* did not occur in these areas, and accordingly, in the beginning of 1911, this Commission was instituted by the Chartered Company to ascertain the transmitting agent.

Nawalia, in the Luangwa Valley, was chosen as the site of the laboratory (Pl. XV, figs. 1-2). Work was commenced at the end of June, 1911, and was continued until April, 1912. Our investigations quickly placed it beyond doubt that *Glossina morsitans* was the vector of the human trypanosome, and further revealed the fact that a considerable percentage of game and of 'wild' *Glossina morsitans* were infected with the same parasite.

In April, 1912, the headquarters of the Commission were removed to Ngoa, on the Congo-Zambesi watershed, in order that experiments might be undertaken to ascertain what influence, if any, was exerted by climatic conditions on the transmission of the trypanosome. Work at Ngoa (Pl. XVI, figs. 1-2) was continued until the end of August, 1912, when the Commission left for England. By this time it had been definitely determined that the relatively low temperatures experienced during the cold season on the plateau were inhibitory to the completion of the developmental cycle of the human trypanosome in *Glossina morsitans*.

Records of the greater portion of the work embodied in this report have been published from time to time in the form of separate papers. As, however, these preliminary communications were necessarily somewhat incomplete, it was considered desirable to collect and correlate all the results in the final report.

We desire to acknowledge our indebtedness to Dr. Aylmer May, Principal Medical Officer, Northern Rhodesia; to Dr. A. F. Wallace, M.O., N. Rhodesia; and to E. A. A. Jones, Esq., Assistant Magistrate, Mpika, for the great assistance they rendered the Commission.

The report was completed at the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.

SECTION I

THE HUMAN TRYPANOSOME

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(*a*) OCCURRENCE OF THE DISEASE

The distribution of human trypanosomiasis, due to infection with *T. rhodesiense*, is comparatively wide. When the parasite was first described,* the Luangwa Valley was the only region known to be implicated, but it has since become apparent that the disease is much more widely disseminated.

In the territory formerly known as North-Eastern Rhodesia, cases have been diagnosed not only in the Luangwa Valley, but also in the districts south of Fort Jameson and to the west of Serenje. It may be mentioned further that this parasite was isolated from wild game in the vicinity of Ngoa, and from a native dog living in a village on the Nyasaland boundary. It is possible, therefore, that sporadic cases of the disease may exist in these localities also, but on this point no definite information can be given.

In North-Western Rhodesia, it would appear that *T. rhodesiense* exists in at least one locality, namely, between Broken Hill and the Anglo-Belgian boundary. The details of a case, due to this organism, and contracted in the district named, have recently been described by Ellacombe.†

It is, of course, occasionally very difficult to ascertain the exact locality in which the infection was acquired, more particularly in native cases, but there are good grounds for believing that those from the districts mentioned were autochthonous.

* Stephens and Fantham, (1910). Roy. Soc. Proc., B, Vol. 83, p. 28. Annals of Trop. Med. and Parasit., IV, p. 343.

† Ellacombe, G. W. Sleeping Sickness Bulletin, 1912, Vol. IV, p. 185.

Beyond the confines of Northern Rhodesia, an instance of infection by *T. rhodesiense* in Nyasaland has been recorded by Stannus and Yorke,† an observation which has been confirmed more recently by the Royal Society's Commission.‡ The parasite has been isolated from a case of sleeping sickness originating in Portuguese East Africa,§, and it is accordingly probable that cases reported from the neighbouring portions of German East Africa are due to the same trypanosome. Finally, indigenous cases of sleeping sickness have recently been diagnosed in Southern Rhodesia, though we are not in a position to state the identity of the organism.

It will be seen, therefore, that *Trypanosoma rhodesiense* is widely spread over South Central Africa, and its distribution is in close association with that of *Glossina morsitans*, which has been shown to be the vector.

(*b*) CLINICAL FEATURES OF THE INFECTION

There is no essential difference between the clinical manifestations of the disease caused by *T. rhodesiense* and that due to *T. gambiense*, except possibly the greater virulence of the former.

In the earlier stages, enlargement of the lymphatic glands is commonly seen, and more or less irregular fever, with the accompanying symptoms of malaise, anorexia, headache, pains in the limbs, &c., is the rule. During the attacks of fever trypanosomes can readily be demonstrated in the peripheral blood, but as a rule are rather scanty. As the disease progresses oedemata of various parts of the body, more particularly the face, are common features, together with emaciation, loss of muscular power, ataxic gait, tremors, difficult speech, loss of memory, and other signs of nervous derangement. These symptoms gradually increase in intensity until finally the patient sinks into a condition of coma, and dies.

No exact data exist as to the duration of the disease in natives, but it would appear to be short. Many of the cases complained of

† Stannus and Yorke. Proc. Roy. Soc., B 84, 1911, p. 156.

‡ Royal Society Commission. Roy. Soc. Proc., B 85, 1912, p. 423.

§ Sleeping Sickness Diary. Nyasaland Protectorate.

no subjective symptoms of the disease when diagnosed, and presented very few objective signs, but in general they lived only a few months. Occasionally, however, a patient is more resistant, and one native definitely proved to be infected with *T. rhodesiense* is still alive and in a state of apparent good health a year later.

(*c*) IDENTITY OF TRYPANOSOME WITH *T. RHODESIENSE*

The essential characteristic of *T. rhodesiense*, Stephens and Fantham, is the occurrence of 'posterior nuclei,' i.e., amongst the short forms of the parasite, organisms in which the macronucleus is markedly displaced from the usual central to a decidedly posterior position are commonly seen in certain laboratory animals, more especially rats. This displacement may proceed to such a degree that the macronucleus may actually be situated behind the micronucleus at the aflagellar end of the trypanosome. As this peculiarity has not been observed in *T. gambiense*, the two strains of human trypanosomes can readily be distinguished. A further difference is noticed in the pathogenicity of the parasites, *T. rhodesiense* being much more virulent for all species of animals than *T. gambiense*.

At Nawalia and Ngoa, rats were subinoculated from sixteen cases of sleeping sickness, and in every instance the posterior nuclei were observed in stained preparations. The pathogenicity of these strains agreed closely with that of the Armstrong strain,* from which *T. rhodesiense* was originally described.

It will be understood, therefore, that in all our transmission experiments the strains of human trypanosomes utilised answered in all respects to the description of *T. rhodesiense*.

(*d*). TRANSMISSION OF THE TRYPANOSOME

Experiments to transmit the human parasite by *Glossina morsitans* were made at Nawalia in the Luangwa Valley, and at Ngoa on the Congo-Zambesi watershed. Nawalia, the site of an old station, is situated on the right bank of the Nyamadzi river,

* Bevan. Journal of Comparative Path. and Therapeutics, 1910, p. 160.
Yorke. Annals of Tropical Medicine and Parasitology, 1910, p. 351.

a tributary of the Luangwa, at an altitude of about 2,100 feet above the sea. Its position is approximately 12° 25′ S. and 32° 2′ E. Ngoa, 11° 40′ S. and 31° 30′ E. lies some 30 miles North of the station of Mpika, at a height of 4,400 feet above sea level.

The meteorological observations at Nawalia and Ngoa are synopsised in Tables 1 and 2.

Certain general conditions which attach to all the experiments may be mentioned.

The identity of the flies has been controlled both by direct examination of the external characters and by the preparation of the male genitalia, as recommended by Newstead,* so that we can state with some degree of confidence that we have been dealing only with *Glossina morsitans*, Westw.

All the experimental animals have been kept in fly-proof cages, the fronts of which were protected by a double layer of wire gauze, the inner composed of coarse, and the outer of mosquito meshing. The two layers were separated by a space of one inch in order to obviate the possibility of an animal being bitten while pressing its body against the front of the cage.

The feeding of the flies, and the changing into fresh bottles daily, was supervised personally, while the flies were kept in such a manner that they had no opportunity of obtaining food from other than the animals used in the actual experiments.

Experiments at Nawalia

A. With Laboratory-bred *Glossina morsitans*

Experiment 1

Commenced August 20th, 1911.

It is somewhat difficult to tabulate this experiment, owing to the fact that it was not started on a definite date with a definite number of flies. Between August 20th and September 29th twenty-six flies had hatched out, and each, as it did so, was given its first meal on an animal showing numerous parasites in the peripheral blood, so that on any given date the periods which had elapsed since the infecting feeds of the flies varied considerably. In Table 3 the main facts in connection with the flies are given.

* Newstead, R. Bull. Entomol. Research, Vol. II, Part 1, May, 1911.

FIG. 1. Camp at Nawalia, Luangwa Valley.

FIG. 2. Laboratory at Nawalia, Luangwa Valley.

Table 1.— Meteorological observations at Nawalia, N. Rhodesia, 12° 25′ S., 32° 2′ E. Altitude 2,100 feet (approximately).

1911-1912	External Shade Temperatures			Laboratory Temperatures			Relative humidity %	Rainfall inches	Days on which rain fell
	Mean	Absolute maximum	Absolute minimum	Mean	Absolute maximum	Absolute minimum			
June	67·2	89·0	44·3	—	—	—	48·6	0	—
July	68·7	91·0	44·6	67·4	82·2	51·8	45·7	0	—
August	73·3	96·8	51·2	71·2	85·5	57·8	35·8	[illegible]	—
September ...	77·5	103·3	54·0	71·5	92·5	61·5	31·5	0	—
October	86·1	107·6	62·3	84·5	99·5	71·2	31·8	[illegible]·26	2
November ...	87·1	107·8	67·9	84·6	96·	74·5	41·1	1·61	8
December ...	82·3	101·7	67·8	79·6	91·5	71·	69·1	8·54	20
January	80·6	101·1	67·0	78·4	87·1	72·8	77·7	14·97	16
February ...	79·2	96·0	66·1	77·1	84·9	71·[illegible]	73·8	5·35	16
March	79·0	97·6	61·0	72·[illegible]	86·8	67·5	62·5	5·10	6
April (to 9th)	79·5	97·9	59·8	77·2	87·7	61·9	58·[illegible]	[illegible]·[illegible]1	1
								36·[illegible]4	69

Table 2.—Meteorological observations at Ngoa, N. Rhodesia, 11° 40′ S., 31° 30′ E. Altitude 4450 feet (approximately).

1912	External Shade Temperatures			Laboratory Temperatures			Relative humidity %	Rainfall inches	Days on which rain fell
	Mean	Absolute maximum	Absolute minimum	Mean	Absolute maximum	Absolute minimum			
April (20th to 30th)	72·0	89·7	54·1	68·0	76·8	59·0	53·7	0	—
May	69·0	90·5	45·1	66·2	77·0	51·0	51·3	0	—
June	61·6	85·5	34·3	60·5	72·5	42·0	53·0	0	—
July	62·4	83·0	42·3	60·9	72·0	49·1	53·0	0·01	1
August	67·7	86·4	45·2	65·7	71·5	60·0	46·7	0	—
								0·01	1

TABLE 3.—Giving date of infecting meal, date of death, and duration of life after infecting meal.

No. of fly	Date of infecting meal	Date on which fly died	Duration of life from date of infection
A 1	20.8.11	22.9.11	33 days
A 2	21.8.11	20.10.11	60 ,,
A 3	25.8.11	6.9.11	12 ,,
A 4	26.8.11	23.10.11	58 ,,
A 5	27.8.11	28.10.11	62 ,,
A 6	27.8.11	17.10.11	51 ,,
A 7	28.8.11	11.10.11	44 ,,
A 8	29.8.11	11.11.11	74 ,,
A 9	30.8.11	12.9.11	13 ,,
A 10	31.8.11	20.10.11	50 ,,
A 11	31.8.11	12.9.11	12 ,,
A 12	3.9.11	27.9.11	24 ,,
A 13	3.9.11	5.9.11	2 ,,
A 14	5.9.11	28.9.11	23 ,,
A 15	6.9.11	25.10.11	49 ,,
A 16	7.9.11	4.11.11	58 ,,
A 17	8.9.11	3.10.11	25 ,,
A 18	8.9.11	14.10.11	36 ,,
A 19	9.9.11	19.10.11	40 ,,
A 20	9.9.11	13.9.11	4 ,,
A 21	13.9.11	23.10.11	40 ,,
A 22	16.9.11	14.11.11	59 ,,
A 23	17.9.11	27.10.11	40 ,,
A 24	25.9.11	3.11.11	39 ,,
A 25	26.9.11	29.9.11	3 ,,
A 26	26.9.11	29.10.11	33 ,,

Twenty-four hours after each fly had fed on an infected animal, it was afforded an opportunity of feeding on a clean monkey (No. 41), after forty-eight hours on a second (No. 42), and from the third day onwards on a third (No. 52). The schedule of feedings is given in Table 4.

TABLE 4.—Showing transmission of human trypanosomes by laboratory-bred *Glossina morsitans*.

Date	Animal	No. flies fed	Result	Remarks
Aug. 21—Sept. 18...	Monkey 41	5*	Negative	Flies fed 24 hours after infecting feed.
,, 22 ,, 28...	,, 42	14*	,,	Flies fed 48 hours after infecting feed.
,, 23 ,, 26...	,, 52	23	Infection	Flies fed 72 hours and onwards after infecting feed.
Sept. 27 and 28 ...	,, 68	5	Negative	(*a*) Infecting feed over 30 days before.
	,, 69	6	,,	(*b*) Infecting feed between 20 to 30 days before.
	,, 70	5	Infection	(*c*) Infecting feed less than 20 days before.
Sept. 29—Oct. 3 ...	,, 72	16	,,	All the flies fed.
Oct. 4	White rat 77	16	,,	All the flies fed.
,, 5	Monkey 68	14	Negative	Infected fly did not feed.
,, 6—9... ...	,, 58	16	Infection	All the flies fed.
,, 9	,, 61	1	,,	Infected fly, only, fed.
,, 11—12 ...	,, 68	14	,,	Infected fly fed on 13th and 14th as well.
,, 13—16 ...	,, 69	13	Negative	Infected fly did not feed.
,, 16—19 ...	,, 83	12	Infection	Infected fly commenced feeding on 16th, others on 17th.
,, 20—28 ...	,, 69	10	Negative	Infected fly did not feed.
,, 29—Nov. 11...	,, 56	4	,,	Infected fly did not feed.

* The remaining flies refused to feed.

From this table it will be seen that neither of the first two monkeys on which the flies were allowed to feed became infected, whereas No. 52 did so on the 27th September.

A reference to Table 3 will show that up to, and including the 26th September, twenty-three flies had fed on an infected animal more than three days previously, and had, accordingly,

been fed on Monkey No. 52. The three flies, A 24, 25 and 26, had never fed on this animal, and therefore had not to be considered in the attempt to isolate the infected fly. Moreover, six of the flies, A 1, 3, 9, 11, 13 and 20, had died prior to the 26th September, and of these, three proved to be negative throughout on examination. The other three, namely, A 3, 11 and 20, were found to show a heavy intestinal infection of trypanosomes. Fly A 3 died on September 6th, A 11 on September 12th, and A 20 on September 13th, while the monkey did not become infected until September 27th, much too long an incubation period for one of these flies to have been the infecting one. We have additional proof for the conclusion in that the abdominal contents (gut + salivary glands) of flies A 3 and A 20, on inoculation into monkeys, did not determine an infection.

On the 26th September there were, then, twenty flies with which to deal, amongst which was at least one infective fly. As stated above, three flies, A 24, 25 and 26, had never fed on Monkey No. 52, so that the inquiry was limited to seventeen, and this was further reduced to 15 by the death of flies A 12 and 14 on September 27th and 28th—both flies negative on examination. These were accordingly split up into three groups, based on the length of time which had elapsed since the date of the infecting feed, and each group was allowed to feed for two days on a clean monkey, Nos. 68, 69 and 70.

Group (*a*) Infecting meal over thirty days previously.

,, (*b*) ,, ,, between twenty and thirty days previously.

,, (*c*) ,, ,, less than twenty days previously.

Of the three monkeys, No. 70 was the only one to become infected, and the transmitting fly was thus located in Group (*c*), consisting of A 19, 21, 22 and 23.

While waiting to ascertain which of the three monkeys would become infected, all the flies were fed from September 29th to October 3rd on Monkey No. 72, and on October 4th on White Rat No. 77. Both of these animals became infected in due course.

On October 5th all the flies, with the exception of A 19, were re-fed on Monkey No. 68, and from the 6th to the 9th all were fed on Monkey No. 58, except on October 9th when fly A 19 alone,

was fed on Monkey No. 61. Of these animals, No. 68 did not become infected, while Nos. 58 and 61 did. The fly A 19 (♂) was thus proved to be the infecting one.

No particular interest attaches to the further experiments. From the table it will be seen that both those animals on which fly A 19 fed became infected (Nos. 68 and 83), whereas those on which it did not feed remained quite healthy (Nos. 69 and 56).

When it had been definitely ascertained which was the infecting fly, it was possible to determine fairly accurately the duration of the cycle of the trypanosomes in the insect. Fly A 19 had its infecting meal on September 9th, and Monkey No. 52, the first to become infected, showed trypanosomes in the peripheral blood on September 27th. On the 26th, therefore, the last day on which the flies were fed on this animal, a period of eighteen days had elapsed since A 19 fed on the infected animal. The average incubation period of the local strain of human trypanosomes in monkeys is four to five days, and subtracting this from the eighteen days, it is evident that the fly must have become infective in thirteen days.

This fly, A 19, lived for forty days from the date of the infecting meal, and between the time of becoming capable of transmitting the parasite and the date of death had fed on eight animals, all of which became infected.

The other flies were fed continuously from the date of the possible infecting meal to that of death, which occurred at varying periods from two to seventy-four days, but none of them became infective.

Experiment 2

Commenced November 14th, 1911, with sixteen laboratory-bred *Glossina morsitans*.

In this experiment the flies were infected directly on a case of human trypanosomiasis, each fly being allowed to feed on one occasion only. Ten fed on the 14th of November, when the patient showed three trypanosomes per field in the blood, and the remaining six on the 15th, when there was, on an average, one trypanosome to seven fields (Zeiss Oc. 4, Obj. D.D.). The subsequent meals were as shown in Table 5.

Table 5. -Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Days after infecting feed	Animal	No. of flies fed	Result	Remarks
1 to 5	White rat 116	15	Negative	
6 to 10	,, 118	15	,,	
11 to 15	,, 124	15	Infection	
16 to 20	,, 116	13	,,	
21 to 22	,, 118	12	,,	
23 to 27	Monkey 137	6	Negative	Flies divided into two groups to separate the infective one.
23 to 27	,, 138	5*	Infection	
28 to 42	,, 148	Varied	Negative	

* One fly of this group (6) refused to feed, and died on the 26th day of the experiment.

Rat No. 124 became infected on December 4th, five days after the flies had fed last, and as the incubation period of the trypanosome in these animals, on an average, is five days, it seems probable that the fifteenth day was the one on which the infecting fly became capable of transmitting the parasite.

On the 7th of December (23rd day after infecting meal) the twelve flies then alive were divided into two groups in order to effect an isolation of the infective one, and were fed on Monkey Nos. 137 and 138, as indicated in the table. On December 12th, the fly numbered B 23 (♂) died, and on examination proved to be heavily infected throughout the alimentary canal, and in the salivary glands. No infection of the proboscis, however, was observed.

The other flies were fed on a clean monkey until the 42nd day, but without result.

Experiment 3.

Commenced December 29th, 1911, with twenty laboratory-bred flies.

These flies were infected directly from a patient in whose peripheral blood three trypanosomes per field (Zeiss Oc. 4,

Obj. D.D.) were seen. They were afterwards fed daily for sixty-five days on a series of healthy monkeys, none of which became infected. From the 67th—70th day of the experiment, the seven flies then alive were fed on a guinea-pig heavily infected with the human trypanosome, and were then fed for a further period of thirty days on a clean monkey. This did not become infected.

Experiment 4

Commenced January 12th, 1912, with twenty-three laboratory-bred flies.

These were fed for four days on a patient showing, on an average, one trypanosome to three fields in the peripheral blood, and afterwards on healthy monkeys, as indicated in Table 6.

TABLE 6.—Showing transmission of human trypanosome by laboratory-bred *Glossina morsitans*.

Day	Animal	No. of flies fed	Result	Remarks
0 to 3	Patient	23	—	
4	—	—	—	Flies starved.
5 to 8	Monkey 237	22	Negative	
9 to 12	,, 238	22	,,	
13 to 16	,, 240	22	,,	
17 to 20	,, 254	20	Infection	
21 to 23	,, 237	18	—	Died on 24th day.
24	,, 240	17	Infection	
25 to 29	,, 260	9	Negative	Flies divided into two groups.
	,, 261	9	Infection	
30 to 60	,, 272	16—0	Negative	Infected fly did not feed.

On February 20th, the 29th day of the experiment, the fly numbered D 18 died, and on dissection was found to show a massive intestinal infection of trypanosomes. Unfortunately, the fly had been dead for some hours before it was examined, and it was found impossible to dissect out the salivary glands. The whole abdominal contents, therefore (gut and glands) were crushed

up in normal saline solution and inoculated into a healthy monkey, which became infected five days later. The disease ran a typical course.

None of the other flies—dissected as they died—were found to harbour trypanosomes in the proboscis, gut, or salivary glands.

In this instance the time which elapsed from the date of the *first* infective meal until the date on which the fly became capable of transmitting the trypanosome (allowing five days for the incubation period in the monkey) was nineteen days.

B. By 'wild' *Glossina morsitans*

Experiment 5

Commenced on November 14th, 1911, with ninety-eight 'wild' flies.

Prior to infecting these flies with the human trypanosome, they were fed for three days, November 14th-16th, on a healthy monkey (No. 95), and for the next four days on a native fowl. The monkey never became infected. From the 21st to the 24th of November the insects then alive, fifty-seven in number, were fed on an infected monkey showing twenty to thirty trypanosomes per field in the peripheral blood, and were afterwards fed on healthy animals, as in Table 7.

Table 7.—Result of feeding 'wild' *Glossina morsitans* on clean monkeys, after a preliminary meal on an animal infected with the human trypanosome.

Days after first infecting feed	Animal	No. of flies fed	Result	Remarks
4 to 6	Monkey 125	48	—	Monkey died on 7th day.
7 to 9	,, 127	41	Negative	
10 to 13	,, 130	34	Infection	
14 to 16	,, 119	31	,,	
17 to 18	,, 140	7	Negative	Monkey died on 19th day.
17 to 23	,, 141	10	Infection	Flies divided into two groups to isolate infected one.
20 to 25	,, 144	4	,,	

This experiment was finished after the flies had fed on the 25th day, the flies being then killed and embedded.

The duration of the cycle of the parasites in the flies, in this instance, would appear to be slightly over eleven days. The first infecting meal was taken on November 21, and Monkey No. 130 showed parasites in the peripheral blood on December 7, a difference of sixteen days. As stated already, the incubation period of this trypanosome in monkeys is about five days, and by subtracting this from the sixteen days, we obtain eleven for the duration of the development cycle.

Experiment 6.

Commenced January 12, 1912, with forty-two freshly-caught flies.

After being fed for one day on a monkey infected with the human trypanosome, and showing numerous parasites in the peripheral blood, the flies were fed on a clean monkey for nine days. They were then starved for one day, and subsequently allowed to feed on clean monkeys and rats from the 11th to the 33rd day. None of these animals became infected. The flies were dissected as they died, and while trypanosomes were found in the gut and proboscis of several, in no instance was an infection of the salivary glands observed.

Experiment 7

Commenced January 12, 1912, with forty-two freshly-caught flies.

The details of this experiment are exactly similar to those of Experiment 6, with the exception that from the 1st to the 9th day the flies were fed on a native fowl instead of on a monkey. They were starved on the 10th day, as before, and afterwards fed on clean monkeys and rats from the 11th to the 38th day. None of these animals became infected. Trypanosomes were found in the proboscis and gut of several of the flies when dissected, but in no case were the salivary glands implicated.

Experiment 8

Commenced February 14th, 1912, with 104 freshly-caught flies.

On the 13th of February, the flies were fed on a healthy monkey

which did not become infected, thus excluding the possibility that they were already infected with the trypanosome. On the four succeeding days they were fed on a guinea-pig infected with the human trypanosome, and showing numerous parasites in the peripheral blood, and afterwards on clean monkeys as indicated in Table 8.

Table 8.—Showing transmission of the human trypanosome by freshly-caught *Glossina morsitans*.

Day	Animal		No. of flies fed	Result	Remarks
4					Flies starved.
5 to 10	Monkey	269	98	Negative	
11					Flies starved.
12	,,	269	64	,,	
13 to 27	,,	280	41	,,	Died on 28th day. Flies divided into two groups, A and B.
13 to 29	,,	281	47	Infection	
28 to 29	,,	286	33	Negative	Group A, only, fed.
30	,,	269	17	Infection	Group B, only, fed.
30 to 38	,,	300	10	Negative	A 1
30 to 40	,,	301	10	,,	A 2 — Flies of group A divided into 3 sub-groups, A 1, A 2, A 3.
30 to 40	,,	302	12	,,	A 3
31 to 33	,,	303	12	,,	B 1
31 to 37	,,	304	11	Infection	B 2 — Flies in group B divided into three sub-groups, B 1, B 2, and B 3.
31 to 34	,,	305	12	Negative	B 3
34 to 39	,,	310	11	,,	Sub-group B 1 fed.
35 to 52	,,	315	12	,,	Sub-group B 3 fed.
39 to 52	,,	300	15	,,	Sub-group A 1 and B 2 fed.
41 to 52	,,	301	28	,,	Sub-group A 2, A 3, and B 1 fed.

The insects were dissected as they died, but only in one, the infective fly, was an infection of the salivary glands observed, though in a considerable number an infection of the proboscis and gut was found.

The duration of the development cycle of the trypanosomes in the fly would appear to be twenty-five days in this experiment. The flies were fed for the *first* time on the infected guinea-pig on February 14th, and the first monkey became infected on March 15th, thirty days later. The average incubation period of the disease in monkeys is five days, so that the cycle took twenty-five days to complete.

Discussion of Results

In these transmission experiments, there are at least three sources of error which must be considered, (1) accidental infection of the experimental animals by other than the experimental flies, (2) hereditary transmission of trypanosomes from infected female flies to their progeny, and (3) natural infection in the experimental animals.

(1) With regard to the first of these, the conditions under which the experimental flies and animals were kept have been mentioned already, and it seems more than improbable that accidental infection would account for the unfailing regularity with which the animals became infected after the infective flies had fed on them. Moreover, in all our experiments, 644, such an occurrence as the unexpected infection of an animal has not been observed.

(2) The number of bred flies which we have been able to obtain has been too small to permit us to examine many of them prior to use in the experiments, but such as were, have been found uniformly free of infection. Stuhlmann,* Kleine,† and Bruce‡ with his colleagues have examined large numbers of bred flies belonging to the species *Glossina brevipalpis*, *Glossina morsitans*, and *Glossina palpalis*, and are unanimous in the opinion that hereditary transmission of trypanosomes does not occur amongst the tsetse flies.

(3) With reference to the third point, we have used in the course some 256 monkeys, and have never seen a naturally-occurring trypanosome infection in any of them. *Plasmodium kochi* and microfilaria have been observed, but beyond these, nothing.

* Stuhlmann. Arbeit aus d. Kaiser. Gesundheitsamte, Band XXVI, Heft 3, p. 374.

† Kleine. Deutsche med. Wochenschrift, No. 45, 1909.

‡ Bruce, Hamerton, Bateman and Mackie. Reports of S.S. Commission of the Royal Society, No. 11, pp. 122-125.

Experiment 2 was specially devised to obviate the possibility of error through the use of local monkeys (*Cercopithecus pygerythrus*). The flies, as they were obtained, were fed on healthy, imported rabbits which showed no signs of infection throughout; they were infected directly from the human host; and they were then fed on white rats. With the exception of Experiment 1, all the bred flies used in the transmission work were infected directly on the human host.

The trypanosomes transmitted by these flies were identical with the human one, both morphologically and in their animal reactions.

There are certain points in connection with the experiments which appear to be worthy of emphasis. The number of bred flies which has been used in each is strikingly small, very much more so than in any other similar work, of which the records are available. In the four experiments a total of eighty-five was employed, and of these three only became infective. A percentage of 3·5.

The time occupied by the trypanosomes in completing their cycle in the flies is also strikingly short, approximately two weeks (thirteen, fifteen, eleven, nineteen and twenty-five days).

It may be pointed out, however, that all our estimations of the latent periods of the trypanosomes in the flies represent the probable durations only. Although the average incubation period in monkeys is five days, this has been found to vary from three to eight days, and it is possible, therefore, that the cycle may have been slightly shorter, or longer, in any one instance.

Moreover, a further source of error is introduced in those experiments in which the flies were fed on an infected animal for more than a single day. It has yet to be determined whether only a definite percentage of flies are inherently capable of transmitting the disease, or whether *any* fly will do so provided that it has an opportunity of feeding on an infected animal at some particular time during its existence. If the latter alternative be correct, the peculiar factors governing their infectability have still to be ascertained. Assuming the first view to be correct, then the latent period of the trypanosomes in the flies must date from the *first* occasion on which the insects were fed on the infected animal, while, if the second be correct, the latent period may date from *any* of the meals on the infected animal.

So far as our results go, we have seen no indication of late infection in any of our flies, although some of them have lived as long as seventy-four days after the potentially infecting meal.*

All our results go to show that mechanical transmission of the trypanosomes does not occur, that is, if a period of twenty-four hours has elapsed since the infecting meal. We have not made any experiments to ascertain whether infection could be accomplished by interrupted feeding. This has been proved with various insects, but practically, would account for very few, if any, cases of the disease.

The infective flies have been found to retain the power of transmitting the parasites during life, and do not require to feed more than a single time on an animal in order to infect it, neither do they require, prior to becoming infected, to feed more than once on an animal suffering from trypanosomiasis.

With regard to Experiment No. 5, although only two flies were definitely proved to transmit the trypanosome, infection of the salivary glands was found, on dissection, in four others. As will be seen in Section V, this is strong presumptive evidence that these four were also infective.

Experiments at Ngoa

A. With Laboratory-bred *Glossina morsitans*

Experiment 9

Commenced on June 23rd, 1912, with nineteen laboratory-bred flies.

They were fed for five days on a heavily-infected guinea-pig, and afterwards for fifty-five days on a healthy monkey which did not become infected.

B. By 'Wild' *Glossina morsitans*

Experiment 10

Commenced on May 18th, 1912, with 116 'wild' *Glossina morsitans*, which had previously been shown to be non-infective by being fed on a healthy monkey. The flies were fed for four days on a heavily-infected guinea-pig and afterwards for sixty-seven days on three healthy monkeys, none of which became infected.

* See, however, page 209.

FIG. 1. Camp at Ngoa, Congo-Zambesi Watershed.

FIG. 2. Laboratory at Ngoa, Congo-Zambesi Watershed.

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Experiment 11

Commenced on June 13th, 1912, with ninety 'wild' flies previously proved not to be transmitting the human trypanosome. They were allowed to feed for three days on a heavily-infected guinea-pig, and were afterwards allowed to feed on a healthy monkey until the 40th day of the experiment. This monkey did not become infected.

On the 41st day of the experiment the flies then alive, forty-two in number, were placed in an incubator, kept at a temperature of 85° F. Three flies were found to be infective eight days later.

Experiment 12

Commenced on June 14th, 1912, with 119 'wild' *Glossina morsitans*, proved to be non-infective. The flies were fed from the 1st to the 3rd day of the experiment on a heavily-infected guinea-pig, and afterwards to the 60th day on a healthy monkey, which did not become infected.

On the 61st day the thirty-eight remaining flies were placed in the incubator kept at 83° F., and were fed from the 61st-75th day on a clean monkey. The monkey died on the 76th day without becoming infected. However, on dissecting the flies, one was found to harbour trypanosomes in the gut and salivary glands, and the contents of these structures inoculated into healthy monkeys caused the animals to become infected with *T. rhodesiense*.

Experiment 13

Commenced on July 11th, 1912, with 176 'wild' *Glossina morsitans*, previously shown not to harbour the human trypanosome. These flies were fed for three days on a heavily-infected guinea-pig, and afterwards from the 5th—51st day on healthy monkeys, none of which became infected.

Experiment 14.

Commenced on July 24th, 1912, with 160 'wild' *Glossina morsitans*, previously proved to be non-infective. From the 1st—4th day they were fed on a heavily-infected guinea-pig, and from the 5th—36th on a healthy monkey, which did not become infected.

(*e*) INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF THE TRYPANOSOME IN *GLOSSINA MORSITANS*

At Nawalia, eight transmission experiments were made, four with laboratory-bred, and four with 'wild' *Glossina morsitans*. At Ngoa, six experiments were carried out, five with 'wild' and one with bred flies. It is unfortunate that the bred flies in the latter series were not more numerous, but owing to the low temperature the majority of the flies did not emerge from the puparia, and many of those which did were malformed, and quickly died. In all the experiments in which 'wild' flies were used, however, the possibility that they were already infected with the trypanosome was excluded by first feeding them on healthy monkeys.

Synopses of the two series of experiments are given in Tables 9 and 10.

It will be seen from these tables that whereas, in the Luangwa Valley, *Trypanosoma rhodesiense* was successfully transmitted by *Glossina morsitans*, all efforts in this direction on the Congo-Zambesi watershed have been in vain. Of 330 flies used in the valley experiments, six, and probably ten, became infective. The larger figure is based on the number of salivary gland infections found in the flies. Our experience indicates that the implication of these structures is intimately connected with the ability of *Glossina morsitans* to transmit *Trypanosoma rhodesiense*, and that until they are invaded by the organisms the flies are non-infective. Salivary gland infections have been found in all the flies which were capable of transmitting the parasite. In Experiment No. 5 (Table 7) six flies were found to harbour trypanosomes in the glands, but of these only two were actually proved to transmit *Trypanosoma rhodesiense*. As in all other instances, it was shown conclusively that those flies in which trypanosomes were found in the salivary glands were infective, it may be concluded that the remaining four flies in this experiment were also capable of transmitting the parasite. Invasion of the salivary glands has not been observed except in those flies which were known to transmit *Trypanosoma rhodesiense*.

In the six plateau experiments, 680 *Glossina morsitans* were employed without a single fly becoming infective.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity* %
1	20.8.11	Dry	26	Bred	Infection	13	75·1	90·0	60·5	35·0
2	14.11.11	Commence-ment of rains	16	,,	,,	15	83·5	93·5	74·5	44·8
3	14.11.11		57	Wild	,,	11	83·8	93·5	74·5	44·8
4	29.12.11	Rainy	20	Bred	Negative	—	78·2	87·1	72·8	74·5
5	12.1.12	Rainy	42	Wild	,,	—	78·0	85·7	72·8	74·2
6	12.1.12	,,	42	,,	,,	—	78·0	85·7	72·8	74·2
7	12.1.12	,,	23	Bred	Infection	19	77·9	85·7	72·8	75·4
8	13.2.12	,,	104	Wild	,,	25	77·3	86·0	71·0	66·5

* In the cases of the unsuccessful experiments, the mean temperature and the relative humidity have been calculated for the first 30 days only.

TABLE 10.—Synopsis of transmission experiments with *T. rhodesiense* and *Glossina morsitans*, carried out at Ngoa, Congo-Zambesi watershed.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity %
1	18.5.12	Dry	116	Wild	Negative	—	62·7	74·5	50·5	52·0
2	13.6.12	,,	90	,,	,,	—	59·1	72·4	42·0	52·0
3	14.6.12	,,	119	,,	,,	—	59·1	72·4	42·0	52·0
4	20.6.12	,,	19	Bred	,,	—	60·2	71·8	42·0	52·0
5	11.7.12	,,	176	Wild	,,	—	62·0	72·0	49·1	50·0
6	24.7.12	,,	160	,,	,,	—	64·7	76·1	51·0	47·0

The explanation of these apparently contradictory results is at first sight not very obvious, more particularly in view of the fact that even on the plateau 'wild' *Glossina morsitans* capable of infecting healthy monkeys with *Trypanosoma rhodesiense* were occasionally encountered. If the climatic conditions under which the valley experiments were carried out be compared with those obtaining during the plateau experiments, it will be seen at once that the most striking difference is one of temperature. As a rule, the temperature during the former series of experiments was roughly from 15-20° F. higher than during the latter series.

With a view to ascertaining the influence, if any, exerted by temperature on the developmental cycle of *Trypanosoma rhodesiense* in the tsetse fly, a further series of experiments were performed on the plateau, in which, by means of an incubator, the flies were kept at a temperature approximating to that of the valley at the most favourable season.

In the first two experiments 'wild' flies were used. No water was placed in the incubator, and the warm dry air was found to have a very deleterious effect on the insects. Within the first seven days, twenty-five of the sixty-one flies with which Experiment No. 1 was commenced, and fifty-three of the seventy-two in Experiment No. 2 had died.

Notwithstanding the small number alive at the end of the second week, two infective flies were obtained in the first experiment and one in the second. In the third experiment laboratory-bred flies were employed instead of 'wild' ones. Attention was drawn to the fact that the low temperatures obtaining at Ngoa in the cold season were very unfavourable to the pupation of *Glossina morsitans;* in fact, so slow was the process that in spite of the large number of pupae at our disposal, we were unable to procure sufficient flies for experimental purposes. The difficulty was all the greater as many of those which did emerge were malformed, and quickly died. In order to obtain a sufficient number of bred flies for this experiment we resorted to the expedient of placing the pupae, some of which had been deposited over two months previously, in the incubator (85° F.). Within three or four days a large number of flies were procured.

The experiment was commenced on August 8th with thirty bred

flies, to which were added twelve on the 9th, eleven on the 10th, and three on the 11th. These groups were fed for four, three, two and one days, respectively, on a heavily-infected guinea-pig and afterwards on a healthy monkey. Parasites were found in the blood of the animal on which the flies of the fourth group were fed on the 18th after the insects fed on the guinea-pig, so that, allowing five days for the incubation of the disease in the monkey, the fly was infective on the 13th day. The monkey on which the flies in Group 1 were fed became infected on the 26th day of the experiment, so that the duration of the developmental cycle of the parasite in the fly would be twenty-one days. As we cannot be certain that the infective fly fed on the infected guinea-pig on each of the four days, the latent period in the insect may be anything from seventeen to twenty-one days. The animals on which Groups 2 and 3 were fed did not become infected.

It will be seen from Table 11 that the total number of flies used in the incubator experiments was 189—133 'wild' and 56 'bred.' Of the 'wild' flies, three became infective, and of the 'bred' two, i.e., 2·6 % of the total number used. This figure is, however, hardly a fair estimate, as of the 133 'wild' flies only seventy-eight were alive at the end of the first seven days of the experiment. This heavy mortality was probably due to the sudden change from the cold external air to the warm, dry atmosphere of the incubator. As a general rule, in our transmission experiments it was found that about 10 % of the flies died during the first week. This was approximately the case in the incubator experiment in which bred flies were used, as, owing to the fact that the insects were hatched out in the incubator they were not subjected to any sudden change of atmospheric conditions. We consider, therefore, that had the mortality of the flies in these two experiments been the customary 10 % instead of over 40 %, the proportion of infective flies would be three of ninety instead of three of 133, or 3·3 % instead of 2·2 %. This figure, 3·3, approximates closely to that obtained in the incubator experiment in which bred flies were used, namely, 3·5, and also to that obtained in the valley experiments, 3·5.

The results of these three series of experiments, viz., those carried out at Nawalia at laboratory temperatures 75°-84° F.,

TABLE II.—Synopsis of experiments to transmit *T. rhodesiense* at Ngoa, Congo-Zambesi Watershed, by means of *Glossina morsitans* kept in incubator.

No.	Date on which started	Season	No. of flies used	Variety of flies used	Result	Duration of developmental cycle days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity %
1	30.6.12	Dry	61	Wild	Infection	14	80·6	87·8	74·5	36·0
2	1.7.12	,,	72	,,	,,	13	80·6	87·8	74·5	36·0
3	8.8.12	,,	56	Bred	,,	13 and 21	82·6	89·2	77·0	72·4

those made in the laboratory at Ngoa at from 59°-65° F., and the incubator experiments at Ngoa 80·6°-82·6° F., show in a most conclusive manner that comparatively high temperatures, 75°-85° F., are necessary for the completion of the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans*.

In addition to temperature, there is another factor in the climatic conditions which might possibly influence the developmental cycle of the trypanosome in *Glossina morsitans*. We refer to the relative humidity of the atmosphere. At the most favourable season of the year in the Luangwa Valley for transmission experiments, and also in the case of the first two carried out in the incubator, the relative humidity was extremely low. In order to decide the point, the relative humidity of the atmosphere in the incubator in the bred fly experiment described above was kept at from 70-72·5%. As the incubation period of the parasite in the flies (twelve and seventeen to twenty-one days respectively) and also the percentage of infective flies obtained (3·5 %) were approximately the same as those in other experiments in which the relative humidity was very low, we can only conclude that this factor does not exert any appreciable influence on the developmental cycle of *T. rhodesiense* in *Glossina morsitans*.

The following experiments were devised with a view to ascertaining more definitely the influence of temperature on the development of the parasite.

Experiment 15

Two batches of 'wild' *Glossina morsitans* (Batch A consisting of 95 and Batch B of 119) in which the possibility of the presence of an infective fly had previously been excluded by feeding the insects on clean monkeys, were fed for three consecutive days on a guinea-pig infected with *T. rhodesiense*. After being starved for a day, each batch was fed on a healthy monkey until the 40th day after the first feed on the infected animal. Neither of the monkeys became infected. Batch A, in which there were forty-two flies still alive, was placed in the incubator, whilst Batch B, in which there were now fifty-eight flies, was kept at laboratory temperature. The sudden change from the laboratory to the warm, dry air of the incubator proved very fatal to the flies

in Batch A, and on the 43rd day only six were alive. From the 41st to the 47th day the flies in this batch were fed on a monkey, and from the 48th day on a rat. The rat became infected on the 53rd day, so that, allowing five days for the incubation of the disease in the animal, Batch A contained an infective fly on the 48th day after the first feed on the infected guinea-pig, and eight days after being placed in the incubator. As the monkey died on the 47th day, we are unable to state whether the fly became infective before the 48th day. The four flies still alive on the 53rd day were fed on four clean rats, and three of these became infected. The monkey on which Batch B was fed was still negative at the end of sixty days, when there were thirty-eight flies alive.

Experiment 16

This is really a continuation of the former experiment. The thirty-eight flies in Batch B were placed in the incubator on the 61st day after the first feed on the infected guinea-pig, and were fed from the 61st-75th day on a healthy monkey (No. 443). Unfortunately the animal died on the 76th day, so that we were unable to determine with certainty whether any of the flies became infective. All the flies were dissected as they died, and one was found to harbour trypanosomes in the gut and salivary glands. Animals inoculated with the contents of these structures became infected with *T. rhodesiense*. As in our experience all flies in which salivary infection was observed were capable of infecting animals with the human trypanosome, we may assume that had the monkey (No. 443) lived a few days longer it would have been found to be infected.

In Experiment 15 the relative humidity of the air in the incubator was very low (36 %), while in Experiment 16 the relative humidity was comparatively high (72 %). In addition to confirming the view that a relatively high temperature is essential to the completion of the developmental cycle of *T. rhodesiense* in *Glossina morsitans*, and that the relative humidity of the atmosphere is not an important factor, these experiments afford more definite information. It is apparent that the earlier stages of the development of the parasite in the fly can occur at comparatively low temperatures (60° F.), and that trypanosomes can persist in this

TABLE 12.—Synopsis of experiments showing effect of raising the temperature on the development of *T. rhodesiense* in *Glossina morsitans*.

No.	Date started	Season	No. of flies used	Variety of flies	Laboratory Conditions				Date on which flies were put in the incubator	No. of flies alive at the time they were first put in incubator	Incubator Conditions				Remarks
					Mean temp.	Absol. max.	Absol. min.	Rel. hum. %			Mean temp.	Absol. max.	Absol. min.	Rel. hum.	
15	13.6.12	Dry	90	Wild	59·1	72·4	42·0	52·0	24.7.12 (41st day)	42	85·0	90·5	77·0	36·0	Three flies became infective on 48th day.
16	14.6.12	,,	119	,,	59·1	72·4	42·0	52·0	14.8.12 (61st day)	38	83·0	91·0	77·0	72·0	One fly became infective about the 72nd day.

stage for at least sixty days. It is obvious that the developmental cycle of the parasite is not complete, since the flies are non-infective, and inoculation of the gut contents into susceptible animals is followed by negative results. For the completion of the cycle it is necessary for the temperature to which the flies are subjected to be raised to a considerable extent (75°-85° F.).

It is interesting to note that the flies in Batch A (Experiment 15) became infective eight days, possibly less, after being placed in the incubator. This is three days less than the shortest incubation period observed in any of our successful transmission experiments, a fact which supports the view that the developmental cycle of the parasite in the fly had proceeded to a certain point at laboratory temperature (60° F.) before the insects were subjected to the higher temperature (80° F.) of the incubator.

The fact that an occasional infective 'wild' fly was encountered on the plateau during a period (May, June and July) when attempts to transmit in the laboratory were invariably unsuccessful requires some explanation. A possible solution may be that the flies in question were infected during the warmer season of the year and had survived into the cold season.

If the results obtained by feeding freshly-caught flies on healthy monkeys in the valley are compared with those from flies caught on the plateau, a marked difference in the number of infections resulting is apparent. In the Luangwa Valley, 3,202 flies were fed in twenty-nine batches, and *Trypanosoma rhodesiense* was isolated in six of the experiments, giving a ratio of 1 infective fly to 534, whereas on the Congo-Zambesi watershed, 5,250 freshly-caught *Glossina morsitans* were fed in groups on forty-one monkeys, with four positive results—1 infective fly to 1,312. As tsetse flies and game are about equally numerous at Nawalia and Ngoa, and as the disease was presumably introduced into the two localities, which are less than seventy miles apart, about the same time, it appears to us that the only essential difference which can account for the fact that the percentage of infective 'wild' flies at Nawalia is two and a half times as great as at Ngoa is the difference in the climatic conditions. It will be seen from Tables 1 and 2 that the temperatures experienced on the Congo-Zambesi watershed during May, June and July, are very much lower than those at Nawalia

FIG. 1. Feeding *Glossina morsitans* on experimental animals.

FIG. 2. Feeding *Glossina morsitans* on experimental animals.

from September to March. It was during the months named that our experiments were carried out at the two places.

Finally, it might be mentioned that the percentage of infective 'wild' flies caught in the valley was greater in the hot than in the cold season. This point is illustrated in Table 13.

TABLE 13.—Percentage of *Glossina morsitans* found infected with *Trypanosoma rhodesiense* at Nawalia at different seasons of the year

1911-1912	Mean external shade temp.	No. of flies fed	No. infective with *T. rhodesiense*	Ratio of infective to non-infective flies
June	67·2	18	0	
July	68·7	385	0	
August ...	73·3	193	0	0 : 790
September ...	77·5	194	0	
October ...	86·1	—	—	
November ...	87·1	270	1	
December ...	82·3	205	0	1 : 338
January ...	80·6	538	2	
February ...	79·2	104	0	
March ...	79·0	823	2	1 : 466
April (to 9th)	79·5	472	1	

The facts brought to light in this investigation afford a satisfactory explanation of some phenomena which have hitherto appeared to be contradictory. Kleine* was unable to transmit *T. gambiense* by *Glossina morsitans* on the Victoria Nyanza (altitude 3,700 feet), whereas Taute† was successful on Lake Tanganyika at a lower altitude (2,680 feet). Although we were unable to transmit *T. rhodesiense* experimentally on the Congo-Zambesi watershed in the cold season, nevertheless we found that a certain percentage of 'wild' flies was infective. This apparent

* Kleine. Deutsche med. Wochenschrift, No. 45, 1909.

† Taute. Reviewed in Bull. S.S. Bureau, No. 31, Nov., 1911.

discrepancy may be explained on the assumption that the flies became infective during the warm season and that a certain number survived into the colder season of the year.

(*f*) THE RESERVOIR OF THE TRYPANOSOME

The possibility that game might act as a reservoir of infection of sleeping sickness areas has been recognised almost since the inception of work on the disease, but up to the present it would appear that the trypanosomes have never been demonstrated in such animals under natural conditions. In Uganda, Bruce, Hamerton and Bateman* have proved that certain species of buck, notably waterbuck, bushbuck, and reedbuck, can readily be infected with *Trypanosoma gambiense* by allowing infected *Glossina palpalis* to feed on them, and that healthy flies, in turn, may be infected from game harbouring parasites in their blood. They were unable, however, to examine a sufficiently large number of head to ascertain whether a natural infection was present.

The importance of the question is obvious, and the results of our investigations on the point afford a striking commentary on the potential danger involved in the infection of the game.

The Luangwa Valley is particularly rich in a widely-varied fauna, and owing to the fact that in the dry season the great bulk of the game tends to collect in the vicinity of the few permanent streams, it has been comparatively simple to shoot buck for the purposes of experimentation. At Ngoa, on the Congo-Zambesi watershed, game is plentiful at certain seasons of the year.

Trypanosomes indistinguishable from *T. rhodesiense* were isolated from buck in both these localities, and, in addition, from one native dog living in a village some fifty miles to the East of Nawalia.

In the Luangwa Valley 127 head of game were examined, and sub-inoculations into healthy monkeys and rats were made from 56. In this manner the human parasite was recovered from the following animals:—

4 Waterbuck,
1 Hartebeest,
2 Mpala,
1 Bushbuck,
1 Warthog.

* Bruce, Hamerton and Bateman. Proc. Roy. Soc., B, Vol. 83, 1911.

On the plateau 124 head were examined, and sub-inoculations were made from sixty. The trypanosome was isolated from two waterbuck only.

There is thus a marked difference in the percentage of game infected with *T. rhodesiense* in the two districts, the parasite being five times as frequent in the valley as in the plateau game.

As the game was shot without discrimination, the figures are probably a fair indication of the proportion of the total game infected in the two areas.

TABLE 14.—Percentage of game infected with *T. rhodesiense*.

NAWALIA (Luangwa Valley)			NGOA (Congo-Zambesi Watershed)		
No. inoculations made	No. infected	%	No. inoculations made	No. infected	%
56	9	16	60	2	3·3

As stated above, the trypanosome was isolated from one native dog only of thirty-five domestic animals examined—cattle, goats and dogs.

Our experience indicates that big game is much the most important reservoir of the infection. During our sojourn in the country some 256 monkeys, 142 wild rats, and 15 wild mice were examined, with negative results. In all, therefore, 698 animals were examined.

Apart from the bigger species of game, most of the smaller wild animals are nocturnal in their habits, and it seems unlikely that many of them would be exposed to infection.

(*g*) OCCURRENCE OF THE TRYPANOSOME IN *GLOSSINA MORSITANS* IN NATURE

Series of experiments were carried out in the Luangwa Valley and on the plateau to determine the species of trypanosome transmitted, in nature, by *Glossina morsitans*.

A number of fly-boys were sent out from time to time to capture and bring into the laboratory 'wild' tsetse flies, which were allowed to feed on healthy monkeys. Full details of these experiments will be found in a later section of this report.

At Nawalia (Luangwa Valley) the human trypanosome was

isolated in six of twenty-nine experiments, in which 3,202 tsetse flies were used. Possibly it was present in a seventh, but as the monkey died on the day after becoming infected, no definite statement can be made other than that the incubation period of the disease was the same as that in known *T. rhodesiense* infections.

At Ngoa (Congo-Zambesi watershed) 5,250 freshly-caught *Glossina morsitans* were fed in forty-two batches on healthy monkeys, and the trypanosome was isolated on four occasions.

The ratio of infective to non-infective flies in the two localities, assuming that only one was capable of transmitting the virus in each instance, is, therefore,

At Nawalia,	At Ngoa,
1 : 534.	1 : 1,312.

No definite comparison can be made between these figures, as the experiments were not carried out under identical conditions. Those at Nawalia were made during both the dry and wet seasons, while those at Ngoa were carried out during the height of the winter at a time when it was impossible to transmit the human trypanosome in the laboratory. However, the difference is so marked that it may safely be concluded that the plateau flies are infective, in nature, to a much smaller extent, than those in the Luangwa Valley.

In one of these experiments the actual infective fly was isolated.

Experiment 17

Commenced October 30th, 1911, with sixty freshly-caught flies, to which were added twenty-two additional ones on the next day. The flies were fed as indicated in Table 15.

On November 13th, the flies still alive, thirteen in number, were killed and embedded. In the sections, numerous parasites were found in the gut and salivary gland of only one of them.

TABLE 15.—Showing the transmission of the human trypanosome by naturally-infected *Glossina morsitans*.

Date	Animal		No. of flies fed	Result	Remarks
Oct. 30—Nov. 4 ...	Monkey	96	60-22	Infection	
Nov. 6	,,	105	29	,,	
Nov. 7—10 ...	,,	108	19	,,	
Nov. 11—12 ...	,,	113	7	Negative	Flies divided into two groups to isolate the infected fly.
Nov. 11—12 ...	,,	114	6	Infection	

(*b*) IDENTITY OF 'GAME' AND 'FLY' STRAINS WITH THE 'HUMAN' STRAIN OF *T. RHODESIENSE*

The conclusion that one of the trypanosomes isolated from game and from naturally-infected tsetse flies is identical with *T. rhodesiense* has been based on a careful study of the morphology and pathogenicity of the three strains in question.

1. *Morphology* (Pl. XVIII)

In fresh preparations, all three strains show the same mixture of short, slowly-moving, and long, active forms, the relative numbers of which vary in the peripheral blood of any animal from day to day.

In stained preparations, it is sufficient to say that it is impossible to distinguish any one of the three strains from the others. Short forms in which the macronucleus lies actually posterior to the blepharoplast have been observed in each of the three strains.

The measurements of the three strains also show an extremely close agreement. Eleven hundred individuals of each have been measured, and the results are given in Tables 16, 17 and 18. The total number of parasites drawn from each variety of laboratory animal is the same in the case of each strain, and only twenty-five have been measured from any one preparation, as it has been found that the average length varies within wide limits, from day to day, in any given animal.

We are of the opinion that the measurement of twenty-five individuals from one preparation gives a fair estimate of the average length of the trypanosome present in the particular blood film, and that the error is less if twenty-five parasites are measured on each of forty-four different days than if, for example, 100 are measured on each of eleven different days.

In measuring the parasites the following technique was adopted:—Thin blood smears, dried in the air, were fixed in absolute alcohol and stained with Giemsa's solution. The trypanosomes were then outlined at a magnification of 2,000 diameters with the aid of an Abbé camera lucida, and the length along the middle line of the body measured by the tangent method described by Stephens and Fantham.*

* Roy. Soc. Proc., B, Vol. 85, p. 223 (1912).

TABLE 16.—Giving details of measurement of 1,100 individuals of the 'human' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	5	6	25	21·03	27·75	15·5
,,	5	11	25	19·5	26·19	13·27
,,	6	8	25	19·41	28·0	13·5
,,	6	15	25	26·3	31·5	19·67
,,	20	9	25	22·3	30·3	17·1
,,	20	13	25	19·97	26·25	13·25
,,	25	9	25	21·28	28·25	18·0
,,	25	13	25	19·57	29·75	15·25
,,	33	8	25	24·2	28·75	16·75
,,	33	9	25	20·59	29·75	15·29
,,	87	11	25	22·81	31·5	18·25
,,	87	21	25	22·41	29·25	18·5
,,	87	22	25	19·95	27·0	17·0
Dog	244	6	25	22·26	29·25	18·75
,,	244	8	25	20·16	24·5	17·5
,,	244	13	25	21·72	31·25	18·25
,,	244	14	25	19·7	22·5	17·25
Rabbit	13	4	25	23·5	30·5	14·5
,,	13	22	25	18·11	24·75	15·5
,,	A	24	25	19·52	39·25	14·5
,,	86	13	25	21·91	29·0	16·75
Guinea-pig	14	14	25	21·09	30·25	17·5
,,	14	20	25	22·03	31·75	16·0
,,	14	22	25	22·21	33·25	14·5
,,	139	25	25	20·66	27·25	15·75
,,	139	36	25	18·2	26·5	14·0
,,	139	52	25	18·4	28·0	13·75
Rat	15	22	25	20·03	25·5	15·75
,,	15	26	25	21·08	28·25	16·75
,,	16	15	25	22·98	33·25	15·70
,,	183	12	25	22·44	30·75	18·75
,,	184	12	25	22·12	31·25	18·0
,,	184	14	25	19·64	31·0	14·25
,,	184	20	25	20·17	27·5	17·0
,,	184	28	25	20·59	30·0	16·75
,,	208	10	25	19·32	23·25	16·75
,,	208	20	25	19·69	24·5	17·0
,,	212	6	25	23·33	31·0	18·75
,,	212	7	25	20·88	32·5	15·5
,,	212	16	25	18·66	22·5	13·75
Mouse	27	12	25	20·95	26·75	18·5
,,	28	6	25	19·94	23·0	17·25
,,	91	6	25	23·94	27·25	18·5
,,	91	10	25	28·65	33·0	21·5
			1100	21·25	39·0	13·25

TABLE 17.—Giving details of measurement of 1100 individuals of 'game' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	71	7	25	24·79	32·9	17·0
,,	71	9	25	19·84	23·8	15·3
,,	99	38	25	26·36	34·25	19·0
,,	120	8	25	20·02	23·5	18·0
,,	120	11	25	21·9	29·25	17·25
,,	120	13	25	17·4	20·0	15·0
,,	130	8	25	25·97	35·5	19·0
,,	130	11	25	22·05	30·5	16·25
,,	199	5	25	22·47	25·75	15·75
,,	199	7	25	23·6	32·25	16·75
,,	201	7	25	23·4	31·0	17·75
,,	201	8	25	21·62	25·5	17·5
,,	201	9	25	19·58	21·75	17·25
Dog, native		?	25	19·1	26·0	15·5
,,	262	5	25	21·69	25·75	18·5
,,	262	7	25	19·13	23·5	13·5
,,	262	11	25	18·34	22·5	16·25
Rabbit	79	11	25	20·02	29·0	15·2
,,	249	9	25	16·18	19·5	13·75
,,	249	13	25	22·29	32·0	15·25
,,	249	13	25	20·91	28·5	15·75
Guinea-pig	251	10	25	20·87	33·25	15·25
,,	251	11	25	22·87	34·5	15·75
,,	251	13	25	23·11	33·75	15·0
,,	251	15	25	23·5	32·25	14·75
,,	251	17	25	24·09	34·25	13·75
,,	251	21	25	21·67	29·75	14·5
Rat	81	14	25	21·05	31·5	16·0
,,	128	20	25	20·25	21·75	17·5
,,	128	22	25	20·3	23·75	16·0
,,	129	?	25	20·9	28·0	16·25
,,	157	21	25	25·65	30·5	14·5
,,	157	42	25	19·27	21·5	16·75
,,	157	49	25	22·8	32·5	16·25
,,	195	26	25	21·8	35·0	16·5
,,	195	36	25	19·0	24·5	17·0
,,	213	17	25	17·38	19·0	14·5
,,	213	26	25	22·31	34·25	17·5
,,	221	7	25	18·91	23·0	16·5
,,	221	14	25	21·91	35·5	11·75
Mouse	176	9	25	20·13	26·5	17·5
,,	176	14	25	20·99	26·5	16·75
,,	178	6	25	22·89	29·5	17.25
,,	178	7	25	21·6	27·0	16·5
			1100	21·38	35·5	11·75

TABLE 18.—Giving details of measurement of 1100 individuals of 'fly' strain.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Monkey	96	7	25	25·7	32·0	16·0
,,	96	8	25	24·8	33·5	16·5
,,	96	9	25	25·6	36·25	16·0
,,	96	10	25	23·3	30·75	15·75
,,	96	11	25	22·6	31·0	15·25
,,	96	14	25	20·3	23·5	16·5
,,	114	27	25	22·0	28·0	16·25
,,	114	32	25	20·9	25·25	18·0
,,	114	41	25	20·8	30·75	15·25
,,	210	8	25	24·66	30·56	17·5
,,	210	10	25	20·29	23·25	18·25
,,	217	9	25	26·03	30·75	22·0
,,	316	9	25	24·69	32·25	17·5
Dog	235	5	25	26·7	33·0	19·0
,,	235	7	25	21·4	28·0	19·0
,,	235	9	25	20·0	28·0	18·25
,,	235	13	25	20·0	21·25	18·5
Rabbit	245	7	25	23·5	29·5	16·5
,,	245	8	25	20·0	28·0	14·5
,,	245	9	25	18·75	27·75	16·25
,,	245	13	25	22·84	30·0	17·0
Guinea-pig	246	13	25	19·87	23·5	16·7
,,	246	15	25	20·88	26·0	16·25
,,	246	18	25	17·63	21·5	13·0
,,	246	19	29	19·0	27·25	16·5
,,	246	20	25	18·95	25·25	14·25
,,	246	21	25	21·0	27·5	15·25
Rat	103	4	25	24·1	30·0	17·0
,,	103	5	25	20·3	30·0	16·5
,,	103	8	25	18·8	30·75	14·5
,,	218	6	25	19·47	24·75	16·5
,,	218	9	25	19·3	29·0	14·5
,,	218	14	25	20·1	22·5	17·0
,,	218	16	25	19·41	26·75	16·0
,,	218	18	25	22·0	30·5	18·0
,,	229	6	25	24·55	29·5	19·0
,,	229	8	25	21·09	29·25	17·0
,,	229	9	25	19·5	21·75	17·5
,,	229	13	25	22·3	29·5	18·5
,,	229	15	25	20·31	22·75	17·0
Mouse	247	4	25	23·1	29·5	19·5
,,	247	6	25	23·66	29·5	19·25
,,	247	9	25	22·6	34·0	18·75
,,	247	14	25	20·91	25·25	17·25
			1100	21·67	36·25	13·0

TABLE 19.—Comparison of the measurements of the 'human,' 'game,' and 'fly' strains.

Strain	Length in microns		
	Average	Maximum	Minimum
'Human'	21·25	39·0	13·25
'Game'	21·38	35·5	11·75
'Fly'	21·67	36·25	13·0

The similarity in the measurements is, perhaps, best appreciated by a glance at the curves obtained by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured (see Chart I).

A comparison of the percentages of 'short and stumpy,' 'intermediate' and 'long' forms is also of interest.

TABLE 20.—Comparison of percentages of 'short and stumpy,' 'intermediate,' and 'long' forms of the 'human,' 'game,' and 'fly' strains.

Strain	Short and stumpy forms 11-21μ	Intermediate forms 22-24μ	Long forms 25-39μ
'Human'	64·78	15·98	19·14
'Game'	62·87	15·34	21·56
'Fly'	58·68	18·81	22·41

2. *Pathogenicity*

The pathogenicity of the three strains is synopsised in Table 21, p. 223. A glance at this table will show how remarkably the three strains agree in this respect.

CHART I. Comparison of curves of the 'human,' 'game,' and 'fly' strains by plotting out the distribution of the various lengths of the parasites, expressed in percentages of the total numbers measured (1,100 of each strain).

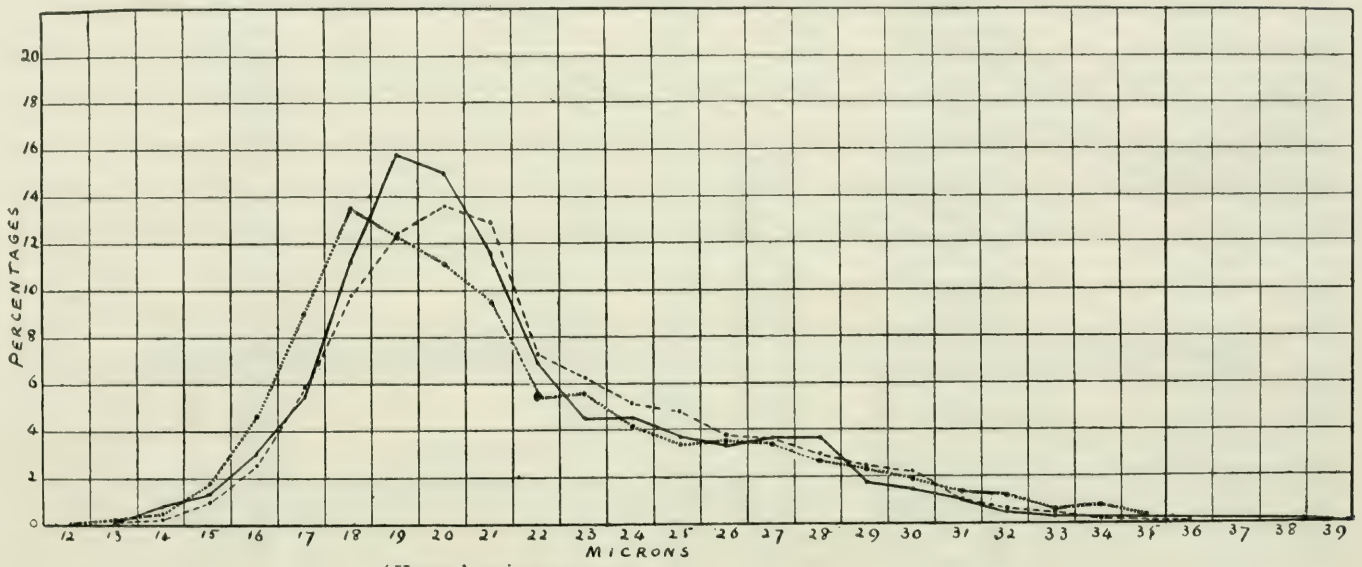

TABLE 21.—Comparison of the pathogenicity of the 'human,' 'game' and 'fly' strains of *T. rhodesiense*.

Animal	'HUMAN' STRAIN			'GAME' STRAIN			'FLY' STRAIN		
	No. used	Incubation days	Duration days	No. used	Incubation days	Duration days	No. used	Incubation days	Duration days
Monkey	47	3—9 Average 5	6—42 Average 18	14	4—11 Average 6	7—63 Average 17	12	4—11 Average 6	7—60 Average 24
Ox	1	7	209	1	7	210	1	7	186
Dog	1	5	26	1	5	25	1	5	26
Rabbit	5	4—6 Average 5	15—62 Average 31	5	4—8 Average 6	14—32 Average 26	5	4—7 Average 5	19—39 Average 29
Guinea-pig ...	6	6—19 Average 11½	48—119 Average 76	5	4—17 Average 11	53—100 Average 72	4	11—17 Average 14	47—127 Average 74 (1 alive after 55 days)
Rat	41	2—8 Average 5	7—83 Average 27½	15	3—8 Average 5	11—87 Average 35	10	2—7 Average 4	9—58 Average 34
Mouse	4	4	15—63 Average 32	2	4—5 Average 4½	48—51 Average 44½	1	4	92
Genet	1	6	76						

SUMMARY

1. The human trypanosome (*T. rhodesiense*) is distributed widely throughout South Central Africa.

2. There is no essential difference between the clinical manifestations of the disease in man caused by *T. rhodesiense* and that due to *T. gambiense*, except possibly the greater virulence of the former.

3. *T. rhodesiense* is transmitted in Rhodesia by *Glossina morsitans*.

4. Approximately 3·5% of the flies may become permanently infected and capable of transmitting the virus.

5. The period which elapses between the infecting feed of the flies and the date on which they become infective varies from eleven to twenty-five days in the Luangwa Valley.

6. Attempts carried out at laboratory temperature on the Congo-Zambesi plateau during the cold season to transmit the human trypanosome by means of *Glossina morsitans* were invariably unsuccessful in spite of the fact that 680 flies were used in these experiments.

7. The developmental cycle of *T. rhodesiense* in *Glossina morsitans* is to a marked degree influenced by the temperature to which the flies are subjected. High temperatures (75-85° F.) favour the development of the parasite, whilst low temperatures (60°-70° F.) are unfavourable.

8. The first portion of the developmental cycle can proceed at the lower temperatures, but for its completion the higher temperatures are essential.

9. The parasites may persist in the fly at an incomplete stage of their development for at least sixty days under unfavourable climatic conditions.

10. These observations afford an adequate explanation of the extremely long latent periods of trypanosomes in *Glossina* which have occasionally been observed by various workers.

11. The relative humidity of the atmosphere has apparently no influence on the development of the trypanosome in *Glossina morsitans*.

12. Mechanical transmission does not occur if a period of twenty-four hours has elapsed since the infecting meal.

13. *Glossina morsitans*, in nature, has been found to transmit the human trypanosome.

14. The chief reservoir of the human trypanosome is the antelope.

15. The results of examination for the human trypanosome of the blood of a large number of monkeys, wild rats and mice were invariably negative.

DESCRIPTION OF PLATE XVIII

Trypanosoma rhodesiense. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-6. *T. rhodesiense*, 'Human strain,' obtained from a monkey inoculated from a case of Sleeping Sickness.

Figs. 7-12. *T. rhodesiense*, 'Fly strain,' obtained by feeding wild *Glossina morsitans* on healthy animals.

Figs. 13-18. *T. rhodesiense*, 'Game strain,' obtained from game.

SECTION II

TRYPANOSOMES OF GAME AND DOMESTIC STOCK

BY
ALLAN KINGHORN
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Within the confines of the Luangwa Valley a numerous and varied selection of game is found, despite the fact that *Glossina morsitans* is everywhere abundant, but, on the contrary, due doubtless to the presence of these insects, domestic stock is extremely scarce, and in many districts non-existent. A few goats are occasionally found, but some evidence exists to show that these animals are not so insusceptible to trypanosomiasis, under natural conditions, as is locally supposed. Dogs are very seldom seen, and the natives themselves recognise the impossibility of keeping them in the midst of 'fly.' Cattle were seen in one village only.

At Ngoa, on the Congo-Zambesi watershed, game is abundant at certain seasons of the year, and goats and dogs are kept in many of the villages. Although tsetse flies are commonly seen near some of these, such domestic stock animals as exist appear to thrive, though trypanosomes were occasionally found in goats.

(*a*) METHODS

In our experience, trypanosomes are more readily detected in a buck's blood by the examination of thin, stained smears than by that of fresh preparations. Except under unusual circumstances, from one to two hours elapsed after an animal had been shot before its heart reached the laboratory, and this, together with the great heat, had a very deleterious effect on the parasites, destroying their motility, and permitting degenerative changes to occur. In several instances in which fresh preparations, made under these conditions, were examined, as well as blood smears

made in the field and afterwards stained, no trypanosomes were found in the fresh blood, whereas they were present in the permanent preparations.

In making smears, it was found advisable to cut the animal's throat immediately it had been shot, and to obtain the blood from one of the arteries. It is claimed that such films have the following advantages:—

1. Clean, uncontaminated preparations are obtained.

2. The trypanosomes have no opportunity of degenerating, and thus stain more sharply.

3. The preparations are permanent.

4. The parasites can be identified more easily in stained, than in fresh preparations. In this particular the examination of thin films has an obvious advantage over that of thick films.

The preparations were dried in the air, fixed in absolute alcohol, and stained with Giemsa.

Owing to the impossibility of obtaining clean sheep and goats, all the game inoculations were made into monkeys and rats, the amount of blood used varying from 1-10 c.cm. It is recommended that, when possible, sheep and goats be used as well, since they are susceptible to most of the pathogenic trypanosomes, whereas animals such as dogs, monkeys and rats are not. This is an important consideration when dealing with such parasites as *T. vivax* and *T. nanum*.

(*b*) EXAMINATION OF GAME AT NAWALIA AND NGOA

A total of 127 head of game, comprising nineteen genera, was examined at Nawalia, and trypanosomes were found by direct examination, by inoculation, or by both methods, in thirty-three.

At Ngoa, 124 buck, belonging to sixteen genera, were examined, and trypanosomes were found in twenty-one—a percentage of 16·9. Details are given in Tables 22 and 23.

It will be seen that parasites were found at Nawalia by direct examination in twenty-six cases, a percentage of 20·4, while at Ngoa trypanosomes were found in the peripheral blood of only sixteen buck—13·0 %. These are high figures for single observations, and it is probable that had several preparations from each

TABLE 22.—Results of examination of game for trypanosomes at Nawalia.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculations
1. Elephant	1	0	1	0	0	0
2. Rhinoceros	1	0	1	0	0	0
3. Hippopotamus ...	1	0	0	0	0	0
4. Zebra	5	0	3	0	0	0
5. Roan	8	1	2	0	0	1
6. Wildebeest	2	0	1	0	0	0
7. Kudu	7	3	3	1	1	4
8. Hartebeest	6	0	1	0	1	1
9. Waterbuck	28	16	14	5	1	17
10. Puku	10	1	6	0	0	1
11. Mpala...	29	1	13	1	1	2
12. Bushbuck	9	4	6	1	2	6
13. Bushpig	4	0	1	0	0	0
14 Warthog	9	0	3	0	1	1
15. Lion	2	0	0	0	0	0
16. Hunting dog ...	1	0	1	0	0	0
17. Giant rat	1	0	0	0	0	0
18. Genet	2	0	0	0	0	0
19. Squirrel	1	0	0	0	0	0
	127	26	56	8	7	33

Table 23.—Results of examination of game for trypanosomes at Ngoa.

Animal	Number examined	Number in which trypanosomes were found in buck's blood	Number inoculations made	Number positive inoculations in which parasites were seen in buck's blood	Number positive inoculations in which no parasites were seen in buck's blood	Total number buck found infected by examination and inoculations
1. Rhinoceros	6	0	3	0	0	0
2. Zebra	17	0	5	0	0	0
3. Buffalo	6	0	3	0	0	0
4. Eland	15	0	12	0	4	4
5. Roan	5	0	3	0	1	1
6. Hartebeest	8	0	4	0	0	0
7. Waterbuck	27	12	15	3	0	12
8. Puku	8	1	6	0	0	1
9. Sitatunga	2	1	0	0	0	1
10. Duiker	9	2	4	0	0	2
11. Klipspringer	2	0	1	0	0	0
12. Warthog	12	0	3	0	0	0
13. Hyaena	2	0	1	0	0	0
14. Caracal	2	0	0	0	0	0
15. Galago	1	0	0	0	0	0
16. Reedbuck	2	0	0	0	0	0
	124	16	60	3	5	21

buck been searched, the percentage of successes would have been much greater. In several instances, only a single trypanosome was found in a film covering the greater part of a slide, and this after a very careful examination extending over two hours.

A more accurate estimate of the percentage of animals harbouring trypanosomes is afforded by considering only those from

which inoculations were made. An analysis of these gives the following figures:—

	Nawalia.	Ngoa.
Number of inoculations made	56 ...	60
Number of positive inoculations in which parasites were found in buck's blood...	8 ...	3
Number of positive inoculations in which no parasites were found in buck's blood	7 ...	5
Number of negative inoculations in which parasites were found in buck's blood ...	6 ...	6
Total number found infected	21 ...	14

These figures show that at least 37·5% (Nawalia) and 23·3% (Ngoa) of the local fauna were infected with trypanosomes. Both *T. vivax* and *T. nanum* have been found in game, and to both these species monkeys and rats are refractory, so that no conclusions can be drawn regarding the presence or absence of these trypanosomes in animals in which parasites were not found in the blood smears. Had sheep and goats been available for inoculation, it is probable that many more buck would have been shown to harbour the two organisms in question. As a conservative estimate, the percentage of game actually infected with trypanosomes in the vicinity of Nawalia might be placed at 50, and at Ngoa 35.

A further point which is brought out in the tables is that different species of buck appear to vary widely in their susceptibility. Amongst the commoner varieties, trypanosomes were never found either by direct examination, or by inoculation in zebra, buffalo, wildebeest and bushpig, and only rarely in roan, hartebeest, puku, mpala and warthog. Waterbuck, eland, bushbuck and kudu were the species found to be most heavily infected.

To a certain extent, perhaps, these differences may be accounted for by the habitats affected by the various species of game. Kudu and bushbuck, and waterbuck to a lesser extent, are usually found in thick cover from which they seldom emerge, and where they are more constantly exposed to the bites of tsetse flies. Mpala, puku and wildebeest are usually found in open country, frequently remaining for the greater part of the day on wide, bare plains, and here the flies are less noticeable than in the bush. Specific

TABLE 24.—Percentages of various species of game found infected with trypanosomes at Nawalia.

Animal	Number examined	Percentage harbouring trypanosomes
Bushbuck	9	66·6
Waterbuck	28	60·7
Kudu	7	57·1
Hartebeest	6	16·6
Roan	8	12·5
Warthog	9	11·1
Puku	10	10·0
Mpala	29	6·9

TABLE 25.—Percentages of various species of game found infected with trypanosomes at Ngoa.

Animal	Number examined	Percentage harbouring trypanosomes
Sitatunga	2	50
Waterbuck	27	44·4
Eland	15	26·6
Duiker	9	22·2
Roan	5	20
Puku	8	12·5

differences in the amount of immunity enjoyed by buck are probably, however, of much greater importance.

In Tables 26 and 27 are given the species of trypanosomes occurring in each animal in which parasites were found. In compiling the tables, information obtained from the result of inoculations, where these were made, has been utilised. This enables a differentiation to be made between such parasites as *T. pecorum* and *T. nanum*, which are morphologically indistinguishable. *T. vivax* has a characteristic morphology, and can thus be identified in blood smears without difficulty.

TABLE 26.—Trypanosomes found in game at Nawalia.

Animal		Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Bushbuck	1	Negative	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
,,	3	*T. multiforme*, sp. nov.	*T. multiforme*, sp. nov.	*T. multiforme*, sp. nov.
,,	4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	5	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
,,	6	Negative	*T. rhodesiense*	*T. rhodesiense*
Waterbuck	1	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	*T. pecorum* and *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
,,	3	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
,,	4	*T. pecorum* or *T. nanum*	,,	*T. nanum*
,,	5	Negative	*T. pecorum*	*T. pecorum*
,,	6	*T. vivax*	Negative	*T. vivax*
,,	7	*T. vivax*	,,	*T. vivax*
,,	8	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
,,	9	*T. pecorum* or *T. nanum* and *T. vivax*	Negative	*T. nanum* and *T. vivax*
,,	10	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	11	(?) *T. rhodesiense*	Animal died day after inoculation	(?) *T. rhodesiense*
,,	12	*T. rhodesiense*	*T. rhodesiense* and *T. pecorum*	*T. rhodesiense* and *T. pecorum*
,,	13	*T. vivax*	No inoculation	*T. vivax*
,,	14	*T. rhodesiense* and *T. vivax*	*T. rhodesiense*	*T. rhodesiense* and *T. vivax*
,,	15	*T. vivax*	No inoculation	*T. vivax*
,,	16	(?) *T. rhodesiense* and *T. vivax*	,,	(?) *T. rhodesiense* and *T. vivax*
,,	17	(?) *T. rhodesiense*	,,	(?) *T. rhodesiense*
Kudu	1	Negative	*T. pecorum*	*T. pecorum*
,,	2	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,,	3	*T. pecorum* or *T. nanum*	*T. pecorum*	*T. pecorum*
,,	4	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
Roan	1	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
Warthog	1	Negative	*T. rhodesiense*	*T. rhodesiense*
Puku	1	*T. vivax*	No inoculation	*T. vivax*
Mpala	1	Negative	*T. rhodesiense*	*T. rhodesiense*
,,	2	*T. pecorum* or *T. nanum*	*T. pecorum* or *T. rhodesiense*	*T. pecorum* and *T. rhodesiense*
Hartebeest	1	Negative	*T. rhodesiense*	*T. rhodesiense*

TABLE 27.—Trypanosomes found in game at Ngoa.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculations into monkeys and rats	Diagnosis
Waterbuck 1	*T. vivax*	No inoculation	*T. vivax*
,, 2	*T. vivax*	,,	*T. vivax*
,, 3	*T. vivax*	,,	*T. vivax*
,, 4	*T. vivax*	Negative	*T. vivax*
,, 5	*T. vivax*	,,	*T. vivax*
,, 6	*T. vivax*	*T. rhodesiense*	*T. vivax* and *T. rhodesiense*
,, 7	*T vivax*	*T. rhodesiense*	*T. vivax* and *T. rhodesiense*
,, 8	*T. vivax*	Negative	*T. vivax*
,, 9	*T. vivax*	*T. pecorum*	*T. vivax* and *T. pecorum*
,, 10	*T. vivax*	Negative	*T. vivax*
,, 11	*T. vivax*	No inoculation	*T. vivax*
,, 12	*T. vivax*	,,	*T. vivax*
Eland 1	Negative	*T. pecorum*	*T. pecorum*
,, 2	,,	*T. pecorum*	*T. pecorum*
,, 3	,,	*T. pecorum*	*T. pecorum*
,, 4	,,	*T. pecorum*	*T. pecorum*
Roan 1	,,	*T. pecorum*	*T. pecorum*
Puku 1	*T. vivax*	Negative	*T. vivax*
Sitatunga 1	*T. tragelaphi*, sp. nov.	No inoculation	*T. tragelaphi*, sp. nov.
Duiker 1	*T. vivax*	Negative	*T. vivax*
,, 2	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*

As would be expected, double infections in game are not uncommon, and several instances of this are recorded in the tables.

No data exist as to the ultimate effect of infection on game. All the animals which were shot appeared to be in perfect condition, and presented no objective signs of disease. Whether

or not buck succumb to trypanosomiasis it is impossible to say, but as they have increased steadily since rinderpest swept through the country, it may be assumed that their tolerance to trypanosomes is very great.

(c) EXAMINATION OF DOMESTIC STOCK

The domestic animals examined, and the species of trypanosomes found in them, are given in Tables 28 and 29.

TABLE 28.—Examination of domestic stock for trypanosomes at Nawalia.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Cow	*T. pecorum* or *T. nanum*	No inoculation	*T. pecorum* or *T. nanum*
,, ...	*T. pecorum* or *T. nanum*	,,	*T. pecorum* or *T. nanum*
Goat ... 39	*T. vivax*	Negative	*T. vivax*
,, ... 94	*T. vivax* and *T. nanum* or *T. pecorum*	,,	*T. vivax* and *T. nanum*
,, ... 202	*T. pecorum* or *T. nanum*	Negative	*T. nanum*
,, ... 258	*T. vivax*	,,	*T. vivax*
Dog	*T. rhodesiense*	*T. rhodesiense*	*T. rhodesiense*
,, ...	*T. pecorum*	*T. pecorum*	*T. pecorum*
,, ...	*T. pecorum*	No inoculation	*T. pecorum*
,, ...	*T. pecorum*	,,	*T. pecorum*
,, ...	*T.* sp. (*montgomeryi* ?)	Negative	*T.* sp. (*montgomeryi* ?)

TABLE 29.—Examination of domestic stock for trypanosomes at Ngoa.

Animal	Trypanosomes found in peripheral blood	Trypanosomes isolated by inoculation into monkeys and rats	Diagnosis
Goat ... 369	*T. nanum* or *T. pecorum*	No inoculation	*T. nanum* or *T. pecorum*
,, ... 375	*T. vivax* and *T. nanum* or *T. pecorum*	,,	*T. vivax* and *T. nanum* or *T. pecorum*
,, 378	*T. vivax* and *T. nanum* or *T. pecorum*	*T. pecorum*	*T. vivax* and *T. pecorum*

The only native village in which cattle were found was Kambwiri's, some forty miles south-west of Nawalia. At present there are only three head, all that are left of a big herd which existed there some four or five years ago. Two of the three appeared to be in good condition when seen, but the headman of the village fully expected to lose them within a few months. The third beast was obviously ill. The cow in which trypanosomes were found at Fort Jameson was bred on the Government Farm, and had never been beyond the limits of the township. Tsetse have never been seen within some miles of the place, but *Stomoxys* is abundant in the kraals, and at certain seasons of the year various species of *Tabanidae* are common.

In several of the villages on the main road from Nawalia to Fort Jameson, a number of goats were found at the end of August, 1911, and again at the beginning of April, 1912, but at the end of that month not a single animal was alive. *Glossina morsitans* was found around all these villages. The four goats mentioned in Table 28 were under observation at Nawalia for a considerable length of time. During this period, parasites were found in the peripheral blood only at rare intervals. Two were rather thin, but not markedly so, and, apart from this, there were no signs of disease. Goat No. 258 was examined at frequent intervals for two months before parasites were first found, while in the others, trypanosomes were seen on the first occasion. Nos. 39 and 258, after having been under observation for nine and four months respectively, died on the road when the Commission left Nawalia, most probably from being over-driven. The other two are still alive, seven and four months after the diagnosis was made.

The dog in which *T. rhodesiense* was found came from a village just on the Nyasaland border. The natives said that it had not been out of the village for over a year previously. As the disease runs an extremely acute course in these animals, there can be no doubt that the dog was infected locally.

(*d*) EXAMINATION OF SMALL VERMIN

It has been suggested that the small vermin might also act as reservoirs of trypanosomiasis. It must be remembered, however, that many of the small vermin of Tropical Africa are nocturnal, and are, therefore, not subjected to the same extent as are the big game to the bites of *Gl. morsitans*. At Nawalia and at Ngoa we examined in all 142 wild rats, 15 wild mice, 1 wild rabbit, 1 giant rat, 1 squirrel, 1 galago, and 2 genet; the results were uniformly negative. Furthermore, it might be remarked that there is no evidence to show that the small vermin are tolerant of the human trypanosome as are the big game. In those which we infected experimentally the disease ran an acute course, and the animals died. If this be the case with the majority of the small vermin they cannot have the same significance as reservoirs of the virus as have the big game, which can probably harbour the parasite for long periods without exhibiting signs of disease.

Not a single case of infection with trypanosomes was found in the 256 monkeys (*Cercopithecus pygerythus*) examined by us, although infection with filaria and *Plasmodium kochi* was common.

The probable explanation of this is that the monkeys during the daytime catch the tsetse fly before the insects have time to feed on them, whereas, on the other hand, they are frequently bitten by mosquitos whilst they are asleep at night. Moreover, it must be remembered that in these animals infection with the human trypanosome runs an acute course, and those animals which contract the disease quickly succumb.

SUMMARY

Trypanosomes are of frequent occurrence in game and domestic stock in North Eastern Rhodesia. As a conservative estimate the percentage of big game infected with trypanosomes pathogenic to man and domestic stock may at Nawalia (Luangwa Valley) be placed at 50, and at Ngoa (Congo-Zambesi watershed) at 35.

At Nawalia six species of trypanosomes were isolated from game

and domestic stock, viz., *T. rhodesiense*, *T. vivax*, *T. nanum*, *T. pecorum*, *T. montgomeryi*, and *T. multiforme;* whilst at Ngoa five species were found, viz., *T. rhodesiense*, *T. vivax*, *T. nanum*, *T. pecorum*, and *T. tragelaphi*.

The results of examination of over 400 monkeys, wild rats and mice were invariably negative.

SECTION III

TRYPANOSOMES FOUND IN WILD *GLOSSINA MORSITANS*

BY

ALLAN KINGHORN

AND

WARRINGTON YORKE

During the sojourn of the Commission at Nawalia and at Ngoa, experiments were undertaken with the object of ascertaining the species of trypanosomes transmitted, in nature, by *Glossina morsitans*, Westw. The flies, as they were brought to the laboratory, were fed on clean monkeys, which were the only animals available for the purpose. Unfortunately, owing to the lack of healthy goats and sheep, no definite conclusions can be drawn as to whether the fly was infected with such species as *Trypanosoma vivax* and *Trypanosoma nanum*, both of which are of common occurrence in game and domestic stock.

At Nawalia, in the Luangwa Valley, freshly-caught *Glossina morsitans* were fed on healthy monkeys from day to day. In all, 3,410 flies were fed in batches, as they were brought to the laboratory, on thirty-three monkeys, but as five of the latter died within two or three days, inferences can only be drawn as to the infectivity of the 3,202 flies fed on the remaining twenty-nine animals. Details of the experiments are given in Table 30.

It will be seen from the table that three species of trypanosomes were isolated, namely, *Trypanosoma rhodesiense*, *Trypanosoma pecorum*, and a third, hitherto undescribed parasite, for which we propose the name *Trypanosoma ignotum*.

TABLE 30.—Result of feeding freshly-caught *Glossina morsitans* on healthy monkeys at Nawalia.

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
1	21.6.11	18	Negative	
4	5.7.11	128	Infection	*T. ignotum, sp. nov.*
7	19.7.11	97	Negative	
28	28.7.11	160	Infection	*T. ignotum, sp. nov.*
35	7.8.11	193	,,	*T. ignotum. sp. nov.*
95	30.10.11	90	Negative	
96	30.10.11	82	Infection	*? T. rhodesiense*
121	14.11.11	98	Negative	
143	4.12.11	100	,,	
100	6.12.11	105	Infection	*? T. rhodesiense*, animal died day after becoming infected
215	13.1.12	41	,,	*T. ignotum, sp. nov.*
210	7-12.1.12	269	,,	*T. rhodesiense*
217	16.1.12	200	,,	*T. rhodesiense*
224	23.1.12	28	Negative	
259	13.2.12	104	,,	
316	20.3.12	101	Infection	*T. rhodesiense*
317	21.3.12	112	,,	*T. pecorum*
326	25.3.12	93	Negative	
329	26.3.12	130	Infection	*T. ignotum, sp. nov.*
330	27.3.12	137	,,	*T. ignotum, sp. nov.*
333	28.3.12	74	,,	*T. rhodesiense*
334	29.3.12	67	Negative	
336	30.3.12	109	Infection	*T. ignotum, sp. nov.*
340	1.4.12	108	,,	*T. ignotum, sp. nov.*
342	2.4.12	52	Negative	
343	3.4.12	85	Infection	*T. rhodesiense* and *T. ignotum, sp. nov.*
348	4.4.12	90	Negative	
353	6.4.12	137	Infection	*T. pecorum* and *T. ignotum, sp. nov.*
94	15.9.11	194	—	Goat; subinoculated monkeys and rats did not become infected

An analysis shows that of the 3,202 flies used, at least nineteen were capable of infecting monkeys. This figure is based on the assumption that, with the exception of Experiments Nos. 343 and 353, each batch contained but a single infective fly. In each of these experiments it is highly probable that at least two infective flies were present, as in the former both *Trypanosoma rhodesiense* and *Trypanosoma ignotum*, sp. nov., were found in the monkey's blood, and in the latter both *Trypanosoma pecorum* and *Trypanosoma ignotum*, sp. nov. The percentage of flies infected with each of the three trypanosomes is given in tabular form.

TABLE 31.—Proportion of wild *Glossina morsitans* infected with *T. rhodesiense*, *T. pecorum* and *T. ignotum*, *sp. nov.*

Species	Number of flies fed	Number of infections	Ratio of infected to non-infected flies
T. ignotum	3,008	10	1 : 300
T. rhodesiense	3,202	6*	1 : 534
T. pecorum	3,202	2	1 : 1600

In our second interim report* one experiment in which *T. rhodesiense* was obtained was inadvertently omitted, so that this parasite was isolated in six, instead of five instances. In addition, Monkey No. 100 became infected four days after the flies had been fed on it. As the animal died the same day, we were unable to decide the species of trypanosome present, but from the short inoculation it is highly probable that the parasite was *T. rhodesiense*. On this assumption the ratio of infected to non-infected flies would be 1 to 455.

At Ngoa, on the Congo-Zambesi watershed, 5,250 freshly-caught *Glossina morsitans* were fed in batches on forty monkeys and two goats. Details are given in Table 32.

* Kinghorn, A., and Yorke, W. A Further Report on the Transmission of Human Trypanosomes by *Glossina morsitans*, Annals of Tropical Medicine and Parasitology, 1912, Vol. VI, p. 269.

TABLE 32.—Results of feeding freshly-caught *Glossina morsitans* on healthy monkeys at Ngoa.

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
2 goats	May	248	Negative	Subinoculated monkeys did not become infected with *T. rhodesiense* or *T. pecorum*
366	23.4.12	223	Infection	*T. ignotum*
367	23.4.12	212	,,	*T. ignotum*
368	23.4.12	211	,,	*T. ignotum*
377	4.5.12	130	Negative	
379	6.5.12	121	Infection	*T. ignotum*
395	16.5.12	124	Negative	*T. ignotum*
404	28.5.12	146	Infection	*T. rhodesiense* and *T. ignotum*
405	29.5.12	109	,,	*T. ignotum*
412	1.6.12	90	Negative	
421	4.6.12	151	Infection	*T. ignotum*
423	6.6.12	166	,,	*T. ignotum*
429	11.6.12	92	Negative	
430	12.6.12	120	,,	
436	14.6.12	145	,,	
439	17.6.12	136	,,	
444	19.6.12	159	,,	
446	21.6.12	85	,,	
456	24.6.12	120	,,	
460	25.6.12	122	,,	
461	26.6.12	116	Infection	*T. rhodesiense*
464	28.6.12	110	Negative	
465	28.6.12	78	,,	
466	29.6.12	84	,,	
469	1.7.12	118	Infection	*T. ignotum*
471	2.7.12	64	Negative	
476	4.7.12	70	,,	

TABLE 32.—*continued.*

Number of Experiment	Date	Number of flies fed	Result	Trypanosomes isolated
481	5.7.12	80	Negative	
485	6.7.12	96	,,	
487	8.7.12	162	Infection	*T. rhodesiense*
502	11.7.12	104	,,	*T. ignotum* and *T. pecorum*
503	12.7.12	168	Negative	
506	13.7.12	121	,,	
511	15.7.12	84	Infection	*T. ignotum*
514	16.7.12	123	Negative	
515	17.7.12	140	Infection	*T. ignotum*
518	19.7.12	135	,,	*T. ignotum*
521	19.7.12	118	,,	*T. ignotum*
524	20.7.12	160	,,	*T. rhodesiense* and *T. ignotum*
559	2.8.12	118	Negative	
560	3.8.12	93	,,	

It will be seen from Table 33 that *T. rhodesiense*, *T. pecorum* and *T. ignotum* were isolated from the plateau flies in the proportion of 1 in 312, 1 in 1312, and 1 in 5250 respectively.

TABLE 33.—Proportion of wild *Glossina morsitans* infected with *T. ignotum*, *T. rhodesiense* and *T. pecorum*.

Species	Number of flies fed	Number of infections	Ratio of infected to non-infected flies
T. ignotum	5,000	16	1 : 312
T. rhodesiense	5,250	4	1 : 1312
T. pecorum	5,250	1	1 : 5250

SUMMARY

T. rhodesiense, *T. ignotum* and *T. pecorum* are transmitted by *Glossina morsitans* in nature, and were obtained by feeding wild freshly-caught *Glossina morsitans* on healthy monkeys.

SECTION IV

DESCRIPTION OF TRYPANOSOMES FOUND

BY
ALLAN KINGHORN
AND
WARRINGTON YORKE

1. *TRYPANOSOMA RHODESIENSE* (Pl. XVIII)

This parasite has been fully dealt with in a previous section, and requires, therefore, no further description. It was isolated from all the cases of Sleeping Sickness, sixteen in number, observed by the Commission. At Nawalia it was found in four waterbuck, two mpala, one hartebeest, one bushbuck and one warthog—16 % of the game from which inoculations were made—and from one native dog. Parasites resembling *T. rhodesiense* were found in blood films made from three other waterbuck, from which no sub-inoculations were made. At least six, and possibly seven, of 3,202 freshly-caught *Glossina morsitans* were found capable of transmitting this organism. At Ngoa the trypanosome was isolated from two waterbuck, and from four of 5,250 freshly-caught tsetse flies.

2. *TRYPANOSOMA VIVAX* (Pl. XIX, figs. 1-8)

At Nawalia this organism was found in eight waterbuck, one puku and three goats, and at Ngoa in twelve waterbuck, one puku, one duiker and two goats.

MORPHOLOGY

(*a*) In fresh preparations it appears as a club-shaped organism, characterised chiefly by the extraordinary rapidity with which it moves across the field.

(*b*) In stained preparations it is seen to be more or less club-shaped, with a long free flagellum. The greatest width is posterior to the nucleus, which is situated about the middle of the body.

TABLE 34.—Measurements of *T. vivax*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Goat ... 39	?	25	24·35	26	22·5
.. ... 39	?	25	23·67	26·25	21·25
,, ... 258	?	25	22·43	25·5	19·75
,, ... 258	?	25	22·74	25·5	21·25
,, ... 258	?	25	23·48	28·25	20·25
,, ... 448	17	25	24·69	28·25	18·75
,, ... 448	18	25	24·5	28·75	20·25
,, ... 448	19	25	23·22	26·5	19·25
		200	23·63	28·75	18·75

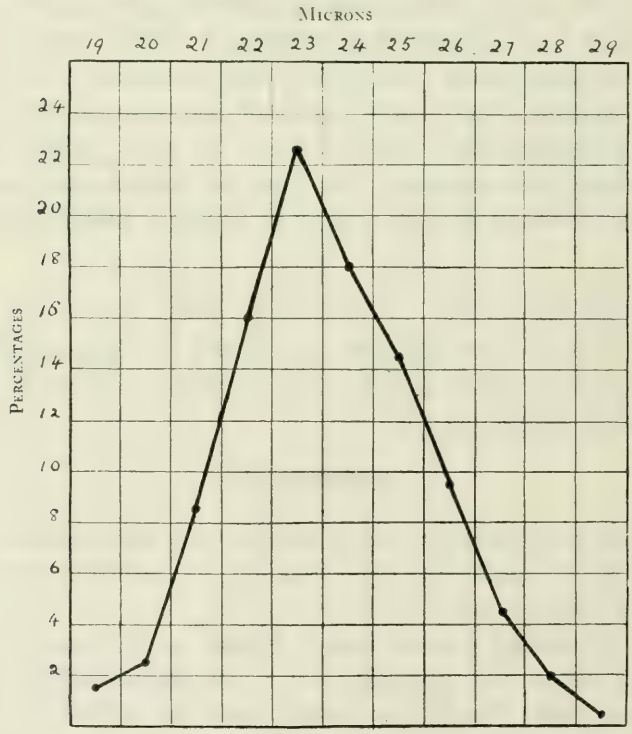


CHART 2.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma vivax*.

The blepharoplast is large and rounded, and lies close to the posterior extremity of the parasite. The undulating membrane is, as a rule, very feebly developed, or absent.

The mean length of 200 trypanosomes was 23·6μ, the maximum 28·75μ, and the minimum 18·75μ (see Table 34). The greatest width varied between 2 and 4·25μ, average 3·2μ.

PATHOGENICITY

Inoculations were made into the following animals:—

8 monkeys	...	...	...	all remained negative.
2 rabbits	...	...	...	both ,, ,,
8 rats ...	...	...	...	all ,, ,,

TRANSMISSION

Owing to the fact that we were unable to obtain a number of clean goats, we could not ascertain definitely that *Glossina morsitans* transmitted this trypanosome in nature. Nevertheless, that this fly can transmit *T. vivax* is shown by the following two experiments. The wild *Glossina morsitans* used for breeding purposes were fed regularly for over two months on goats which were naturally infected with *T. vivax* and *T. pecorum*. These goats were obtained from Ngoa, in the vicinity of which tsetse flies were abundant.

Experiment 1

On July 3rd, forty-eight of the breeding flies, which had previously been starved for five days, were fed on a young, healthy goat. The goat's blood had been examined regularly for ten days before the commencement of the experiment, and no parasites were found. Ten days later the animal became infected with *Trypanosoma vivax*.

Experiment 2

Three other flies were fed on a healthy kid on June 24th, and twelve days later *T. vivax* appeared in the peripheral circulation. The insects were dissected on the day after they had fed on the goat, and trypanosomes were found in one only. The infection

was confined to the proboscis, in which the parasites were extremely numerous and disposed in large rosettes.

Both these goats were brought, in mosquito-proof cages, from a fly-free area.

3. *TRYPANOSOMA NANUM* (Pl. XIX, figs. 9-16)

Found in the following animals at Nawalia:—One bushbuck, three waterbuck and two goats. Possibly it was also present in two other bushbuck, one other waterbuck, two kudu, a roan and two cattle, but as no sub-inoculations were made, it was impossible to differentiate it from *Trypanosoma pecorum*. At Ngoa the parasite may have been present in a duiker and two goats, but in the absence of sub-inoculations it could not be distinguished from *T. pecorum*.

MORPHOLOGY

(*a*) In fresh preparations it appears as a short, sluggish organism. As a rule, it does not progress.

(*b*) In stained preparations it is found to be short, with a more or less rounded posterior extremity from which the body tapers forwards to the acute anterior end; the nucleus is placed at the centre of the body. The blepharoplast is small, and is situated near the posterior extremity. There is no free flagellum, and the undulating membrane is absent, or, at most, very slightly developed. The protoplasm is free of vacuoles and granules, in general.

The mean length of 200 individuals was 14·3μ, the maximum 19μ, and the minimum 10μ (see Table 35). The breadth, at the level of the nucleus, varied from 1 to 2·25μ, the average being 1·5μ.

PATHOGENICITY

The following animals were inoculated:—

3 monkeys	...	...	...	all remained negative.	
1 rabbit	...	...	...	,,	,, ,,
3 rats	...	...	...	...	,, ,, ,,

TABLE 35.—Measurements of *T. nanum*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Goat ... 202	Naturally infected	25	15·2	19·0	12·25
,, ... 202	,,	25	14·77	18·5	11·5
,, ... 202	,,	25	14·0	17·0	11·5
,, ... 202	,,	25	14·61	18·0	11·5
,, ... 202	,,	25	13·99	17·0	11·5
,, ... 202	,,	25	13·51	18·0	10·5
,, ... 202	,,	25	14·43	18·75	10·0
,, ... 202	,,	25	14·39	17·0	12·0
		200	14·36	19·0	10·0

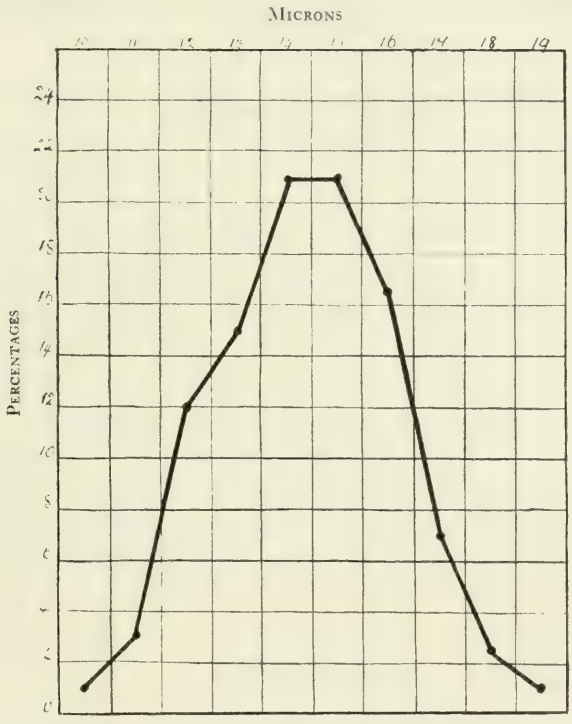


CHART 3.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma nanum*.

DESCRIPTION OF PLATE XIX

Trypanosoma vivax and *Trypanosoma nanum*. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. vivax*.

Figs. 9-16. *T. nanum*.

TRANSMISSION

The vector of *T. nanum* has not been determined with certainty.

4. *TRYPANOSOMA PECORUM* (Pl. XX, figs. 1-8)

At Nawalia this parasite was found in one bushbuck, one mpala, four waterbuck, two kudu, three dogs and one wild rat. It was possibly present in two other bushbuck, a fifth waterbuck, two other kudu, a roan and two cattle, but as mentioned before, it could not be distinguished from *T. nanum* in the absence of sub-inoculations. It was also isolated from 2 of 3,202 freshly-caught *Glossina morsitans*.

At Ngoa it was obtained from one waterbuck, four eland, one roan and one goat. It was possibly present in one duiker and two other goats. It was also found in one wild tsetse fly.

MORPHOLOGY

(*a*) In fresh preparations, this parasite resembles *T. nanum* very closely. It is a short, sluggish organism, showing no degree of translatory power.

(*b*) In stained preparations it appears as a short organism, with an obtuse or rounded posterior extremity from which it tapers to the attenuated anterior end. The nucleus is oval or rounded, and situated near the middle of the body. The blepharoplast is small and rounded, and lies close to the posterior extremity. The undulating membrane, if present, is very feebly marked, and there is no free flagellum. The general protoplasm stains uniformly, and is devoid of granules and vacuoles.

The mean length of 500 individuals was 13·6μ, the maximum 19μ, and the minimum 9·5μ (see Table 36). The breadth, at the level of the nucleus, varied from 1 to 2·5μ, the average being 1·5μ.

PATHOGENICITY

A synopsis of the pathogenicity is given in Table 37, p. 253.

TABLE 36.—Measurements of *T. pecorum*.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Ox	393	11	25	13·73	18·25	10·25
,,	393	14	25	12·92	15·5	9·75
Monkey	2	21	25	13·27	16·25	10·0
,,	9	9	25	13·0	16·8	10·3
,,	37	29	25	14·47	16·75	11·0
,,	109	27	25	13·37	16·75	10·5
,,	109	36	25	13·09	15·75	10·75
,,	123	28	25	15·26	19·0	12·0
,,	317	43	25	14·28	17·5	11·0
,,	317	47	25	13·54	17·5	11·0
,,	339	14	25	15·61	17·75	11·75
Dog	274	(?) Naturally infected	25	14·09	16·75	11·0
Guinea-pig	31	21	25	12·33	15·5	9·5
Rat	49	9	25	13·17	16·0	9·8
,,	163	20	25	13·26	15·25	10·75
,,	163	23	25	13·13	16·25	10·75
,,	282	5	25	12·38	14·75	9·5
,,	282	11	25	13·75	16·0	10·25
Mouse	11	12	25	13·4	16·0	10·6
,,	12	5	25	14·1	16·8	9·8
			500	13·6	19·0	9·5

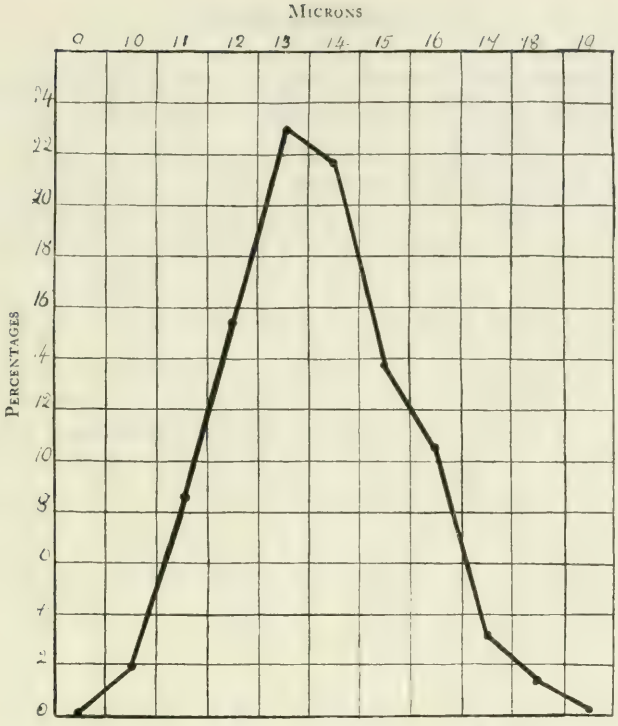


CHART 4.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma pecorum*.

TABLE 37.—Pathogenicity of *Trypanosoma pecorum*.

Animal	No. inoculated	Incubation period days	Duration days
Monkey	17	8-21, average 12½	23-225 (still alive)
Rabbit	3	8-11, ,, 10	66-215
Guinea-pig	1	5	39
Rat	14	3-14, average 6½	6-32
Mouse	2	5	27-75
Goat	3	11-18, average 15	53-58 (still alive)
Ox	1	9	

TRANSMISSION

T. pecorum was obtained in two of twenty-eight experiments in which freshly-caught *Glossina morsitans* were fed on clean monkeys at Nawalia, and in one of forty experiments at Ngoa. In all, therefore, 3 of 8,452 wild flies were found to be naturally infected.

At Ngoa batches of breeding flies which had fed for two to three months on goats, naturally-infected with *T. pecorum*, were allowed to feed on three healthy goats. That on which the first batch, consisting of thirty flies, were fed became infected with the parasite on the 11th day of the experiment. Trypanosomes were found in the blood of the second goat, on which eight flies were fed, on the 12th day. The third goat, on which three flies were fed, became infected after an incubation period of eighteen days. The flies in the last two groups were dissected the day after they had fed. Trypanosomes were found in the gut and proboscis of a number, but in no instance was an infection of the salivary glands observed.

These experiments afford satisfactory proof that *Glossina morsitans* transmits the parasite in nature.

5. *TRYPANOSOMA MULTIFORME*, sp. nov.
(Pl. XX, figs. 9-16)

This parasite was isolated from a bushbuck at Nawalia.

MORPHOLOGY

(*a*) In fresh preparations, the trypanosome is seen to be markedly polymorphic, resembling in this particular *Trypanosoma rhodesiense*, *Trypanosoma gambiense* and *Trypanosoma pecaudi*. Short sluggishly-moving forms are seen, as well as long free-flagellated, active ones. As a rule, the short varieties are more or less stationary, while the long ones progress fairly rapidly. The proportions of each of the forms varies widely, from day to day, in any one animal.

(*b*) In stained preparations, every gradation between extremely short, aflagellar forms, indistinguishable from *T. pecorum*, and long, slender, free-flagellated parasites are seen. In general, the

trypanosome morphologically closely resembles *T. gambiense* or *T. brucei*, except for the presence of occasional *pecorum*-like forms.

The mean length of 1,000 individuals was 21·18μ, the maximum 33·5μ, and the minimum 10·5μ. The curve (Chart 5, p. 259) representing the distribution of the various lengths of the trypanosomes, expressed in percentages of the total number measured (1,000) differs from that of *T. gambiense* in that the apex occurs at 17μ.

TABLE 38.—Measurements of *T. multiforme*, sp. nov.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Monkey 166	47	25	18·26	26·0	14·25
,, 166	62	25	16·2	18·5	13·75
,, 166	78	25	15·34	18·0	12·0
,, 312	12	25	17·93	24·75	10·75
,, 312	13	25	16·84	23·0	13·75
,, 312	19	25	18·09	27·5	15·75
,, 312	42	25	19·65	28·0	17·25
,, 312	23	25	24·73	30·25	13·0
,, 312	44	25	17·48	28·0	13·0
,, 360	9	25	23·73	30·75	15·0
,, 360	14	25	18·88	24·5	14·75
,, 360	19	25	20·22	27·75	15·5
Rabbit 370	7	25	18·36	28·25	11·75
,, 370	15	25	24·1	29·75	16·25
,, 370	16	25	20·55	29·0	13·25
,, 370	81	25	23·49	28·75	19·5
,, 414	5	25	25·82	33·5	14·0
,, 414	12	25	25·76	31·0	15·5
,, 414	13	25	23·42	30·0	12·0
,, 414	16	25	21·1	29·25	10·5

TABLE 38.—*T. multiforme*, continued.

Animal	Day of disease	Number measured	Length in Microns		
			Average	Maximum	Minimum
Rabbit 414	18	25	21·49	28·5	13·75
,, 414	22	25	26·03	32·25	18·25
,, 414	71	25	26·41	31·5	20·0
,, 414	78	25	25·84	30·5	17·25
,, 414	79	25	23·8	29·5	12·0
,, 414	81	25	21·44	28·75	15·0
Rat 219	7	25	21·75	31·25	23·75
,, 219	13	25	24·79	32·0	16·25
,, 219	32	25	20·32	26·25	15·75
,, 219	42	25	18·54	26·0	14·0
,, 219	43	25	20·0	30·0	14·25
,, 219	44	25	16·61	23·25	12·75
,, 219	45	25	16·08	25·75	12·75
,, 253	12	25	25·38	33·0	15·75
,, 253	13	25	22·11	33·25	15·75
,, 253	19	25	21·46	30·25	13·25
,, 361	11	25	20·97	32·25	17·25
,, 361	21	25	21·64	30·0	16·0
,, 361	40	25	19·23	30·0	15·25
,, 361	53	25	17·24	19·5	12·5
		1,000	21·18	33·5	10·5

TABLE 39.—Pathogenicity of *Trypanosoma multiforme*.

Animal		Incubation period days	Duration days	Remarks
Monkey	166	8	94	Tryps. last seen on 78th day.
,,	312	10	74	Tryps. last seen on 55th day.
,,	360	7	156	Tryps. last seen on 65th day.
,,	455	4	Still alive 110th day	Tryps. last seen on 16th day.
Rabbit	370	6	119	Tryps. last seen on 112th day.
,,	414	—	—	Did not become infected.
		5 after 2nd inoculation	69	Tryps. present at time of death.
Guinea-pig	371	—	—	Did not become infected. Inoculated twice.
,,	413	—	—	,, ,,
,,	454	—	—	,, ,,
Rat	219	7	104	Tryps. last seen 44th day.
,,	253	12	125	Tryps. last seen 86th day.
,,	361	10	67	Tryps. last seen 61st day.
Ox	391	—	—	Did not become infected. Inoculated twice.
Goat	501	—	—	,, ,,

DIAGNOSIS

This parasite is at once distinguished from *T. pecaudi* and *T. rhodesiense* by the absence of posterior nuclear forms and by its slight pathogenicity for laboratory animals. It more closely resembles *T. gambiense* than any other known species, and is not easily distinguished from this parasite. A study of the biometric curves of the two trypanosomes shows certain differences, and if the percentages of short, intermediate and long forms of each be compared, as in Table 40, the difference is more clearly brought out, the parasite in question exhibiting very few intermediate forms.

TABLE 40.—Comparison of percentages of 'short and stumpy,' 'intermediate,' and 'long' forms of *T. multiforme* and *T. gambiense*.

	Short and stumpy 10—21μ	Intermediate 22—24μ	Long and slender 25—33μ
Trypanosoma multiforme ...	55·6	12·5	31·9
Trypanosoma gambiense ...	51·2	23·1	25·7

The extreme chronicity of the disease caused by this parasite in laboratory animals is an additional point of distinction from *T. gambiense*. We conclude, therefore, that the parasite is a new species, and in view of its marked polymorphism we propose for it the name *Trypanosoma multiforme*.

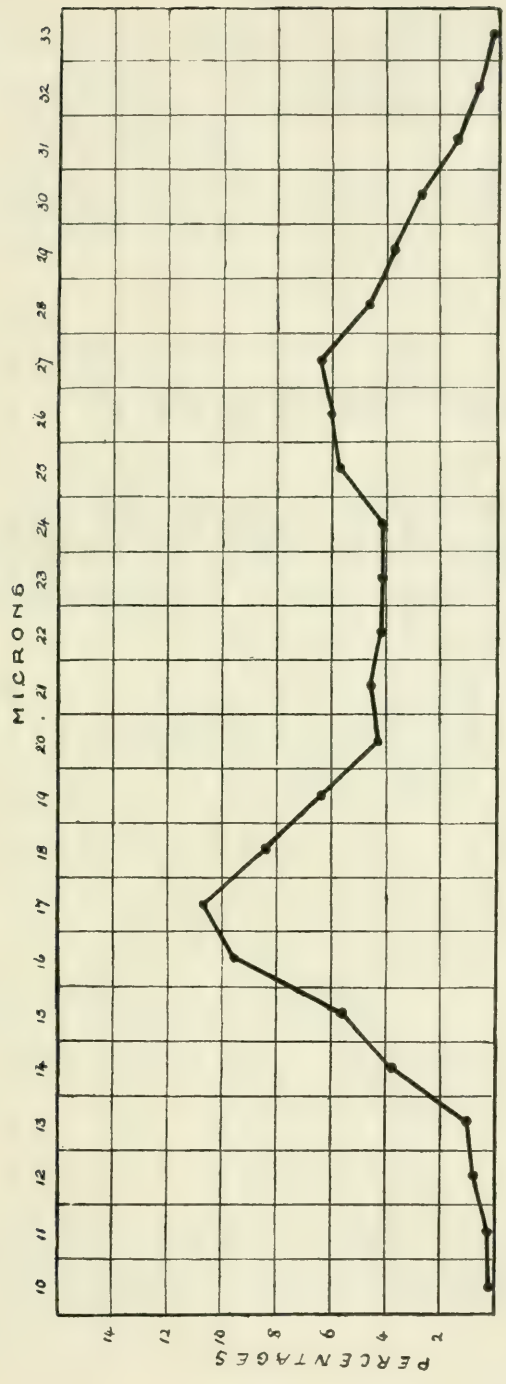


Chart 5.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma multiforme*.

DESCRIPTION OF PLATE XX

Trypanosoma pecorum and *Trypanosoma multiforme*. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. pecorum*.
Figs. 9-16. *T. multiforme*.

TRANSMISSION

The vector of *T. multiforme* is unknown.

6. *TRYPANOSOMA SP.* (*? MONTGOMERYI*)
(Pl. XXI, figs.9-16)

Found in one dog, which was obtained from a village in the hills on the Nyasaland border.

MORPHOLOGY

(*a*) In fresh preparations, the parasite appears as a broad, stumpy organism, which shows no marked degree of translatory power.

(*b*) In stained preparations, the trypanosome resembles at first sight *T. pecorum*, but on closer examination it can be readily distinguished from this parasite by its great breadth. The ratio of the breadth to the length is 1 : 4·8. The posterior extremity is subacute or rounded, the anterior attenuated. The greatest width lies at the level of the nucleus, which is situated at the middle of the body, or posterior to it. The blepharoplast is very large and rounded, and is situated near the posterior extremity. Frequently it lies at one edge of the trypanosome, and projects laterally as a small excrescence. The undulating membrane is, as a rule, absent, and when present is simple, and feebly developed. Occasionally a short free flagellum, 1-2μ in length, is to be seen, but this is generally absent. The cytoplasm often contains coarse granules and vacuoles. The latter are most commonly seen in the posterior portion of the body, whereas the granules may be scattered generally throughout the protoplasm.

The average length of 200 individuals was 15·88μ, the maximum 20μ, and the minimum 10μ. The breadth, at the level of the nucleus, varied from 1·25-6·5μ, the average being 3·29μ.

PATHOGENICITY

One rat was sub-inoculated from the dog, and had not become infected up to the 13th day afterwards, when it was accidentally killed. Unfortunately the dog died some days previously, so that the strain was lost.

TABLE 41.—Measurements of *T. montgomeryi*.

Animal	Day of disease	Number measured	Length in microns		
			Average	Maximum	Minimum
Dog 206	Naturally-infected 27.1.12	25	15·56	18·25	12·5
.. 206	,, 28.1.12	25	15·83	20·0	12·5
.. 206	.. 29.1.12	25	15·53	18·5	11·75
.. 206	.. 30.1.12	25	16·88	19·25	14·0
.. 206	.. 31.1.12	25	16·84	19·0	14·0
.. 206	.. 1.2.12	25	14·72	17·75	11·5
.. 206	.. 3.2.12	25	15·20	17·75	10·0
.. 206	,, 4.2.12	25	16·55	18·75	13·5
		200	15·88	20·0	10·0

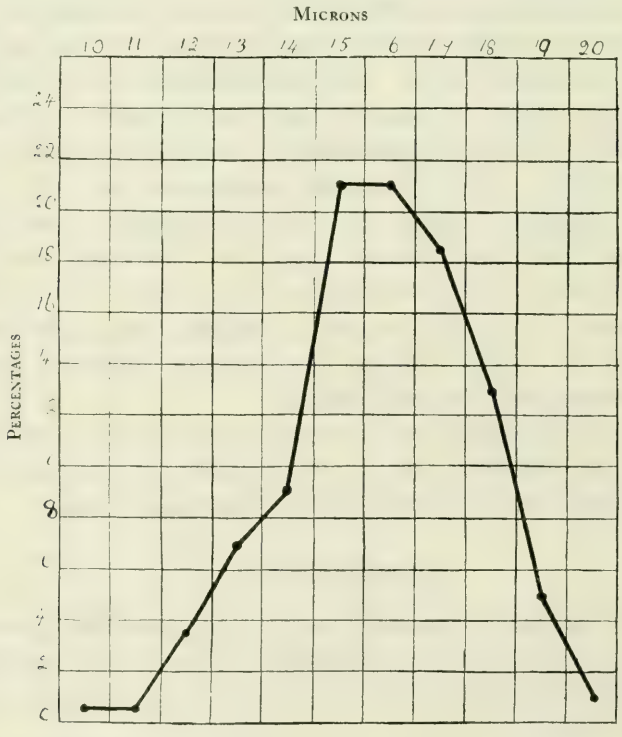


CHART 6.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma montgomeryi*.

TRANSMISSION

The vector of *T. montgomeryi* (?) is unknown.

DIAGNOSIS

It will be seen that, morphologically, this parasite differs widely from *T. pecorum*. The average length is greater, 15·88μ, as compared with 13·6μ. The most characteristic difference, however, is the great width of the organism, which is, on an average, 2·2 times that of *T. pecorum*: average breadth 3·29μ, as compared with 1·5μ. It appears to resemble most closely the parasite described by Montgomery and Kinghorn* as the Ninamwenda strain, and for which the name *Trypanosoma montgomeryi* was proposed by Laveran. But in view of the fact that we were able to examine material from one dog only, we cannot regard its identity with this parasite as established.

7. *TRYPANOSOMA IGNOTUM*, sp. nov. (Pl. XXI, figs. 1-8)

This parasite was obtained by feeding with *Glossina morsitans* on monkeys on ten occasions, and assuming that only one fly was infective in each instance, the proportion of infective to non-infective flies is 1 to 300, or 0·3 %.

MORPHOLOGY

(*a*) *Fresh preparations.* The parasite appears as a comparatively short slender organism. It is fairly actively motile, but exhibits no marked degree of transitory power.

(*b*) *Stained preparations.* The trypanosome is a slender organism of moderate length. The posterior extremity is obtuse, or bluntly rounded, while the anterior is effilated. The nucleus is rounded or oval, and lies at the middle of the body. The blepharoplast is small and rounded, and while usually situated near the posterior extremity, may be separated from it by an appreciable interval. It frequently projects from the edge of the parasite, forming a bud-like excresence. The undulating membrane is feebly developed, and a short free flagellum is only very occasionally present. As a rule, the cytoplasm shows no granules or vacuoles.

The average length of 500 individuals was 16·8μ, the maximum 23μ, and the minimum 12μ (see Table 42).

* Montgomery and Kinghorn, Annals of Trop. Med. and Parasit., Vol. III, No. 2, 1909.

TABLE 42.—Measurements of *T. ignotum*.

Animal		Day of disease	Number measured	Length in microns		
				Average	Maximum	Minimum
Goat	451	17	25	15·55	18·25	13·25
Monkey	1	8	25	16·67	20·25	13·5
,, ...	22	10	25	17·04	20·25	13·75
,, ...	22	11	25	17·99	20·0	15·5
,, ...	36	6	25	16·55	20·5	13·0
,, ...	230	9	25	17·38	23·0	13·5
,, ...	250	11	25	16·52	19·0	13·25
,, ...	363	9	25	17·1	20·0	12·0
,, ...	469	7	25	17·45	19·75	13·75
,, ...	469	8	25	17·19	19·5	13·0
,, ...	469	9	25	16·83	19·25	13·75
,, ...	518	12	25	16·99	19·0	14·75
,, ...	521	9	25	17·19	19·75	14·5
,, ...	521	10	25	16·87	20·25	14·5
Rabbit	44	37	25	16·9	19·25	13·75
,, ...	452	18	25	16·7	20·25	13·25
,, ...	452	20	25	15·43	19·5	12·5
,, ...	452	21	25	15·93	20·75	14·0
,, ...	497	13	25	16·88	21·0	14·0
,, ...	497	25	25	16·93	19·5	13·25
			500	16·8	23·0	12·0

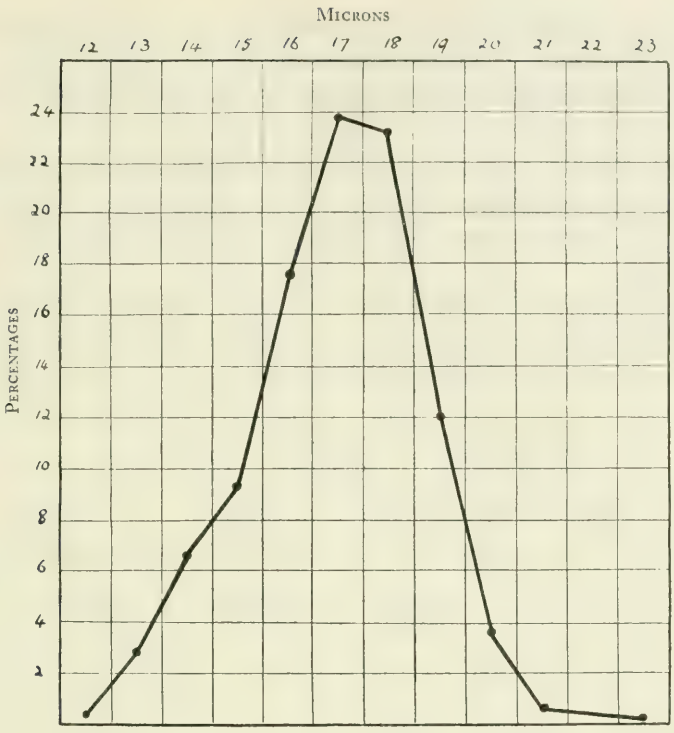


CHART 7.—Giving the curve representing the distribution, by percentages, in respect of length, of *Trypanosoma ignotum*.

PATHOGENICITY

In monkeys, the virulence of *Tryp. ignotum* was found to be very great. The disease is of a fulminating character, the parasites increasing rapidly in number until the animal's death. The trypanosome is of equal virulence in those animals infected directly by the bites of the tsetse flies and in those cases where the strain was passed from monkey to monkey. The incubation period varied from three to ten days, the average being seven, and death occurred two or three days after the parasites appeared in the peripheral blood.

It is interesting to note that on reaching the plateau three monkeys failed to become infected when inoculated with the valley strain of the parasite. At Nawalia, a failure was not recorded, and

it is difficult to understand those mentioned, more particularly as the parasite is of frequent occurrence in the plateau flies, and monkeys infected with this strain react in the same manner as those infected by the valley strain.

Three rabbits were successfully infected with the strain by sub-inoculations from a monkey. The incubation period varied from 13 to 22 days, and the duration of the disease 66 to 106 (still alive).

Two guinea-pigs, twelve rats, four mice, an ox, a goat and a dog were found to be refractory. Moreover, negative results were obtained by feeding infective flies on rats.

TABLE 43.—Pathogenicity of *Trypanosoma ignotum*.

Animal	Number used	Incubation days	Duration days	Remarks
Monkey	36	3—10 Average 7	5—16 Average 14	Three did not become infected.
Rat	12	—	—	None became infected.
Guinea-pig ...	2	—	—	Did not become infected.
Rabbit	3	13—22 Average 17	16—106	Two alive after over 100 days
Mouse	4	—	—	Did not become infected.
Ox	1	—	—	,, ,,
Goat	1	17	68 Still alive	Tryps. only seen once. Monkey inoculated on 55th day did not become infected.
Dog	1	—	—	Did not become infected.

DIAGNOSIS

Morphologically the parasite appears to be distinct from any hitherto described species. The graph (Chart 7) showing the distribution of the trypanosomes in respect of length resembles very closely that of *Trypanosoma uniforme*, but the parasites are at once distinguished by the fact that whereas *Trypanosoma uniforme* is

invariably furnished with a free flagellum,* this, as mentioned above, is of rare occurrence in *Trypanosoma ignotum*, sp. nov. Moreover, the difference between the two trypanosomes is clearly demonstrated by reaction of sub-inoculated animals. According to the Royal Society Commission, *Trypanosoma uniforme* is innocuous to monkeys, an observation which has been confirmed by Fraser and Duke,† who record that they were unable to infect these animals by sub-inoculation from game harbouring the parasite, although goats were readily infected. However, the fact that a large number of monkeys, and one rabbit, quickly succumbed to the disease indicates clearly that the two parasites are not identical.

No information is at present available regarding the alternative host of the trypanosome. Although the parasite has been isolated from wild *Glossina morsitans* much more frequently than any other trypanosome, it has never been found in game or domestic stock. Nothing resembling it has been seen in the peripheral blood of any animal examined in the Luangwa Valley and on the Congo-Zambesi watershed. These include 250 wild animals (elephant, rhinoceros, hippopotamus, buffalo, eland, zebra, wildebeest, roan, kudu, hartebeest, true waterbuck, Crawshay's waterbuck, puku, mpala, bushbuck, duiker, klipspringer, bushpig, warthog, lion, hunting dog, caracal, galago, squirrel, genet, giant rat and rabbit), 35 domestic animals (cattle, goats and dogs), 256 monkeys, 142 wild rats and 15 wild mice—making a total of 698. Eighty-six monkeys have been sub-inoculated from game and domestic animals, and in no instance has an infection with this trypanosome been observed.

In view of the fact that we have been unable to find the vertebrate host, we propose to name the parasite *Trypanosoma ignotum*.‡

*Reports of Sleeping Sickness Commission of the Royal Society, No. XI, 1911, pp. 160-164.

†Fraser, Capt. A. D., and Duke, H. C. Bull. S.S. Bureau, Vol. IV, No. 36, April, 1912, pp. 151-152.

‡ On returning to England at the conclusion of the work of the Commission, we found that the Royal Society Commission had published an account of a parasite which they called *T. simiae*. This is almost certainly identical with *T. ignotum*. As the paper of the Royal Society Commission appeared a few days before our paper on *T. ignotum*, this name must be dropped in favour of *T. simiae*, should the two eventually prove to be identical. (W.Y.)

DESCRIPTION OF PLATE XXI

Trypanosoma ignotum and *Trypanosoma montgomeryi.* Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-8. *T. ignotum.*

Figs. 9-16. *T. montgomeryi.*

TRANSMISSION

In one experiment the infective fly was determined to be one of a group of ten. These were then killed and dissected. Nine of the flies were found to show no trypanosomes in the gut, proboscis, salivary glands or sucking stomach, whereas in the tenth a heavy infection of the proboscis was encountered. The gut, salivary glands and sucking stomach were negative. This observation would indicate that the development of the trypanosome, *T. ignotum*, occurs in the proboscis.

8. *TRYPANOSOMA TRAGELAPHI*, sp. nov. (Pl. XXII)

This parasite was found in blood films made from a sitatunga (*Tragelaphus spekei*) shot near Mpika.

MORPHOLOGY

It is at once distinguished from all known mammalian trypanosomes, with the exception of *T. ingens*, to which it bears a very close general resemblance—so close, in fact, that in a previous report we referred to it by this name.

It is a long, fairly broad trypanosome with a well-marked undulating membrane, sometimes terminating in a short free flagellum. The posterior extremity is effilated. The nucleus appears as a broad band lying transversely across the centre portion of the body, and stains faintly with Giemsa. The blepharoplast is small but distinct, and is situated slightly posterior to the nucleus. The length of the five specimens seen in the single film examined were respectively 52·5, 53, 66, 70 and 72·5 microns, whilst the breadth at the level of the nucleus was 6, 8·5, 5, 6 and 7 microns.

DIAGNOSIS

Whether or not this parasite is *T. ingens*, we are, from the small amount of material available, unable to state with certainty. The general resemblance of the two trypanosomes is striking, but if the camera lucida drawings of this parasite be compared with those of *T. ingens* published in the report of the Royal Society Commission,

it will be seen that the trypanosome in question is shorter and more slender than *T. ingens*, and that the undulating membrane is broader and more pronounced. In the original description of *T. ingens* the length of five specimens are given as 72, 77, 82, 88 and 122 microns. The breadth is stated to be from 7 to 10 microns. It will be thus seen that *T. ingens* is on an average considerably longer than the parasite in question. The average length of the specimens of *T. ingens* measures 88 microns, whereas that of the specimens of this parasite is only 63 microns. We propose *T. tragelaphi* as a name for this parasite.

TRANSMISSION

Unknown.

This infected animal was one of a herd which lived in a large swamp formed by the Luitikila river, about four miles from Mpika.

The sitatunga only emerge from the swamp in the late evening and very early morning for an hour or two in order to feed, and even then do not wander more than a few hundred yards from the water.

Glossina morsitans have never been found within a radius of fifteen miles, and it is therefore exceedingly improbable that they are the vector.

Leeches are found in enormous numbers in the swamp, and feed voraciously on human beings. It seems possible that these animals are the vectors, more particularly as the parasite bears a very close resemblance to amphibian trypanosomes.

Mosquitos are also extremely plentiful in this swamp. Filaria were seen in the blood films of the same animal.

DESCRIPTION OF PLATE XXII

Trypanosoma tragelaphi. Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of the camera lucida at a magnification of 2,000 diameters.

SECTION V

ON THE DEVELOPMENT OF *T. RHODESIENSE* IN *GLOSSINA MORSITANS*

BY

ALLAN KINGHORN, WARRINGTON YORKE

AND

LLEWELLYN LLOYD

In the course of our investigations, we endeavoured to accumulate information regarding the development of the human trypanosome in *G. morsitans*. Reference has already been made to this subject in a previous paper,* and it is here intended to correlate the facts at our disposal. It may be remarked at once that owing to the comparatively small number of laboratory-bred *G. morsitans* available, the information we have collected is by no means so definite as could have been desired.

Up to the present, comparatively little work has been done on this subject, and the records are more or less contradictory. Kleine,† who was the first investigator to write on the development of *T. gambiense* in *G. palpalis*, is of the opinion that the complete cycle takes place in, and is limited to, the intestine, whereas the Royal Society Commissioners‡ in Uganda consider that involvement of the salivary glands is essential. They state that without invasion of the salivary glands there is no infectivity of the fly.

TECHNIQUE

The method of dissection of the flies used by us was that described by one of us in a previous paper. Briefly, it consists in splitting the dorsum of the thorax longitudinally, and, after separating the muscles and loosening the tissues with needles,

* Kinghorn and Yorke. Annals of Tropical Med. and Parasitology, 1912, Vol. VI.

† Kleine. 'Trypanosomenstudien.' Arb. aus d. kaiserl. Gesundheitsamte, Bd. XXXI, Heft 2.

‡ Bruce, etc. Reports of the Roy. Soc. Commission in Uganda, 1911.

drawing out the salivary glands attached to the pharynx through the waist. This method has obvious advantages over that described by the Royal Society Commission, in which after snipping off the terminal segment of the abdomen the whole contents were expressed on to a glass slide, and the salivary glands subsequently separated from the mass of intestines and other structures. We claim for the technique adopted by us that the process is quicker, more certain, and that the danger of contamination from the intestines is reduced to a minimum. In fact, the only lesion in the alimentary canal accompanying the operation occurs in the anterior portion of the oesophagus.

To a certain point the information obtained from our dissections is exceedingly definite. We found that in every fly capable of infecting animals with the human trypanosome (*T. rhodesiense*) the salivary glands were invaded. Of the 160 laboratory-bred *Glossina morsitans* utilised in various experiments to transmit *T. rhodesiense*, 132 were dissected as they died. The remaining twenty-eight were too dry when discovered to allow of dissection. Twenty-seven of those dissected were found to be infected with trypanosomes. The day of the experiment on which the flies died and the results of dissection are given in Table 44.

A glance at the table shows that, of these 132 flies, five became capable of infecting animals with the human trypanosome. In each of these there was an enormous invasion of the salivary glands by trypanosomes. In the 127 flies which remained incapable of transmitting the parasite, the salivary glands were not involved, although trypanosomes were found in the intestines of twenty-two.

A precisely comparable state of affairs was observed on dissection of 'wild' *Glossina morsitans* which had become infective after feeding on infected animals. The salivary glands were found to be infected only in those insects which were capable of transmitting the human trypanosome. In all, 906 'wild' *Glossina morsitans* were used in these experiments, and of this number 620 were dissected. The remainder were for various reasons too dry to admit of dissection. Of these 620 flies the salivary glands of fourteen were invaded by trypanosomes. All except four of these were definitely proved to transmit the human trypanosome. In the case of the other four, the animal upon which the flies had been

TABLE 44.—Results of dissection of laboratory-bred *Gl. morsitans* which were found to contain parasites after being fed on infected animals.

No.	Date of infective feed	Day of dissection after infective feed	Results of Dissection			Remarks
			Proboscis	Intestine	Salivary gland	
1	9.9.11	4	O	+++	O	
2	10.8.12	4	O	+	O	
3	22.1.12	5	O	+	O	
4	9.8.12	5	O	++	O	
5	10.8.12	5	O	+++	O	
6	23.6.12	5	O	+	O	
7	8.8.12	6	O	+++	O	
8	9.8.12	6	O	+++	O	
9	8.8.12	7	O	+++	O	
10	23.6.12	7	O	+	O	
11	10.8.12	8	O	++	O	
12	8.8.12	9	O	+	O	
13	10.8.12	10	O	+++	O	
14	8.8.12	11	O	+++	O	
15	25.8.11	12	O	+++	O	
16	31.8.11	12	O	+++	O	
17	8.8.12	13	O	+++	O	
18	5.3.12	15	+	+++	O	
19	11.8.12	19	++	+++	+++	Infective on 12th day
20	10.8.12	20	+	+++	O	
21	9.8.12	21	2 tryps. seen	+++	O	
22	9.8.12	21	O	+++	O	
23	8.8.12	22	1 tryp. seen	+++	O	
24	8.8.12	22	+	+++	+++	Infective on 17th to 21st day
25	14.11.11	28	O	+++	+++	On 15th
26	22.1.12	29	O	+++	+++	On 19th
27	9.9.11	40	O	+++	+++	On 13th

N.B.—O = Negative, + = Scanty, ++ = Considerable numbers, +++ = Swarming.

allowed to feed died before a diagnosis could be made. None of the 606 flies in which the salivary glands were not involved were able to infect animals with the human trypanosome. Again, the infectivity of *Glossina morsitans* in nature was examined, as mentioned in a previous section, by feeding batches of freshly-caught flies on healthy monkeys. Certain of the groups infected monkeys, and from one of these infective groups the actual infective fly was isolated. This, on dissection, was found to have the salivary glands swarming with trypanosomes. The remaining 242 flies in this group, which had been shown to be non-infective when fed on monkeys, were dissected, and in no instance was an infection of the salivary glands observed.

TABLE 45.—Results of dissection of wild *Gl. morsitans* found to be capable of infecting animals with *T. rhodesiense*.

No.	Date of infective feed	Day of dissection after infective feed	Result of Dissection			Remarks
			Proboscis	Intestine	Salivary glands	
1	21.11.11	25	O	+++	+++	Infective on 11th day.
2	21.11.11	25	O	+++	+++	Infective on 11th day.
3	1.7.12	28	O	+++	+++	Infective on 13th day.
4	21.11.11	30	+	+++	+++	Not proved to be infective.
5	21.11.11	30	+	+++	+++	
6	21.11.11	30	O	+++	+++	
7	21.11.11	30	+	+++	+++	
8	14.2.12	39	O	+++	+++	Infective on 25th day.
9	4.10.11	40	O	+++	+++	Infective in nature.
10	30.6.12	42	O	+++	+++	Infective on 14th day.
11	30.6.12	47	O	+++	+++	Infective on 14th day.
12	13.6.12	58	O	+++	+++	Infective on 48th day after infected meal, or 8 days after having been placed in the incubator.
13	13.6.12	58	O	+++	+++	
14	13.6.12	59	O	+++	+++	
15	14.6.12	71	O	+++	+++	Not proved to be infective, but inoculation of trypanosomes from gut and salivary glands followed by positive results.

In all, twenty *Glossina morsitans* were found to have invasion of the salivary glands by trypanosomes, and of these, sixteen were definitely found to be capable of infecting animals with *T. rhodesiense*. Owing to unavoidable circumstances, we were unable to prove the point in the case of the remaining four, but there is no reason to doubt that had the animals on which these flies were fed survived beyond the necessary five or six days they would have proved to be infected.

In order to anticipate the criticism that the trypanosomes were not really inside the salivary glands, but lying outside these structures, and due to contamination from the gut, our examinations were conducted with extreme care. In the first place, the glands were removed uninjured and attached to the pharynx, placed on a microscope slide, and gently covered with a coverslip. By careful focussing, it could easily be decided that the parasites were actually in the lumen of the tubes and not outside. Moreover, they were usually present in such enormous numbers as absolutely to exclude the possibility that they were the result of contamination from the intestine. Again, the glands of other flies were removed with care and immediately fixed, and subsequently imbedded and cut. In the sections the parasites could be seen to be inside the glands. Finally, in order to remove any possibility of doubt, sections of the whole abdomen of these infective flies were made, and the glands found to be loaded with trypanosomes.

It will be seen from Table 44 that it was by no means a rare occurrence for trypanosomes to be present in the intestines in the earlier stages, especially in the case of those flies examined within a few days of the infected meal. As a general rule, however, most of the insects dissected after the first five or six days were negative. In a certain proportion multiplication of the parasites took place in the intestine.

As to the reason for this multiplication in the gut of occasional flies only, and as to the manner in which it occurs, we have obtained but little information. On one occasion a fly, which died on the 12th day after having been fed on a guinea-pig infected with *T. rhodesiense*, was found to have an enormous gut infection. Possibly there were also a very few trypanosomes in the salivary glands, but on this point we could not be absolutely certain, as the

insect had been dead for some time before the dissection was made. In the mid-gut were found a number of cysts containing swarms of trypanosomes. Some of the cysts had thin walls and were filled with a seething mass of flagellates, while others had thicker walls and the contents were quiescent. The cysts ranged in diameter from 27 to 32μ. Unfortunately, we are unable to state whether the fly was infective at the time of death. It had refused to feed for two or three days previously, and the animal on which it had last fed (9th day of the experiment) did not become infected. The gut contents were inoculated into a monkey, but the animal died from some unknown cause a couple of days later.

Although multiplication of the parasites occurred in the guts of a proportion of the flies, we met no instance in which a fly was infective and in which inoculation of the gut parasites into experimental animals gave rise to infection, unless there was an accompanying invasion of the salivary glands. On the other hand, it appears that on every occasion on which the salivary glands are involved the trypanosomes, both in these structures and also in the intestines, are virulent, i.e., the fly infects when fed on a healthy animal, and inoculation of the parasites from either the salivary glands or the intestine gives rise to infection.

The results of inoculation of trypanosomes from laboratory-bred flies in different stages of infection, and also from the wild flies which were proved to be infective, are given in Table 46.

Our knowledge of the manner in which the salivary glands become infected is uncertain, but there is a certain amount of evidence which would cause one to believe that it is secondary to the intestinal infection, and that it only occurs when the trypanosomes in the gut have reached a certain stage of development, and only then when the conditions of temperature are suitable for the further development of the parasites. In the first place, of 752 flies dissected at various intervals after having fed on infected animals, we never found trypanosomes in the salivary glands in the earlier stages before the flies were infective. Again, whenever trypanosomes were found in the salivary glands there were also enormous numbers present in the intestine. Moreover, it is significant that whenever trypanosomes were found in the salivary glands they were always infective, as were also those present in the gut.

No.	[illegible] animal the day before the fly was dissected	dissected after the infective feed	Proboscis	Intestine	Salivary glands	Result of inoculation of trypanosomes from different portions of the fly into clean monkeys or rats
1	Negative	4	O	+++	O	Gut contents; monkey not infected.
2	,,	5	O	+	O	,, ,,
3	,,	8	O	++	O	,, ,,
4	,,	9	O	+	O	,, ,,
5	,,	10	O	+++	O	,, ,,
6	,,	11	O	+++	O	,, ,,
7	,,	12	O	+++	O	,, ,,
8	,,	13	O	+++	O	,, ,,
9	Positive from 12th day onwards	19	++	+++	+++	Proboscis contents; monkey infected. Gut contents; rat infected. Salivary gland contents; monkey infected.
10	Negative	21	2 tryps. seen	+++	O	Gut contents; monkey not infected.
11	,,	21	O	+++	O	,, ,,
12	,,	22	1 tryp. seen	+++	O	,, ,,
13	Positive from 17th-21st day onwards	22	+	+++	+++	Gut contents; monkey infected. Salivary glands contents; monkey infected.
14	Positive from 15th day onwards	28	O	+++	+++	Gut contents; monkey infected. Salivary glands used for embedding.
15	Positive from 19th day onwards	29	O	+++	+++	Gut contents; monkey infected. Salivary gland contents; monkey infected.
16	Positive from 13th day onwards	29	O	+++	+++	Hind gut contents; rat infected. Fore gut contents; monkey infected. Salivary gland contents; rat infected.
17	Positive from 8th day after putting in incubator and 48th day after first infective feed onwards	58	O	+++	+++	Gut contents; rat infected. Salivary gland (right) contents; monkey infected. Salivary gland (left) contents; monkey infected.
18	?*	71	O	+++	+++	Gut contents; rat positive. Salivary gland contents; rat positive.

* Owing to the unfortunate death of the monkey on which this fly was fed we were unable to ascertain whether the insect was infective or not. The fly was one of the series which was kept for 60 days after the infective feed at laboratory temperature, and on the 61st day placed in the incubator. In view of the fact that the parasites in both the salivary glands and in the intestine were infective to subinoculated rats, it is highly probable that had the animal, on which the fly was fed, lived long enough, it would have been found to be infected.

Attention has already been drawn in a previous section to experiments which suggest that although the parasites can multiply and develop up to a certain stage in the intestine at comparatively low temperatures (55°-65° F.), yet the flies do not become infective until the temperature to which they are subjected is raised to at least 75°-80° F. In none of our experiments were trypanosomes found in the salivary glands of flies which had not been subjected to the higher temperatures. Probably the salivary glands become invaded by parasites which have reached a certain stage in their developmental cycle in the intestine. The remarkably short period (eight days or less) in which three flies, which had been kept forty days after the infective feed at laboratory temperature, became infected after being placed in the incubator at 85° F. can be best explained on the assumption that some portion of the cycle must have occurred in the gut during the first forty days at laboratory temperature.

Whilst the evidence that invasion of the salivary glands is secondary to that of the intestine is fairly conclusive, our knowledge of the path by which the parasites reach the glands from the intestine and of the morphological characteristics of the forms which migrate is by no means so definite.

It is at once apparent that there are two alternative routes by which the trypanosomes might reach the salivary glands from the gut, viz.:—(i) By way of the proboscis; (ii) by penetrating the gut wall, and after crossing the coelom traversing the wall of the salivary glands. We have accumulated no evidence enabling us to decide along which of these two routes the parasites migrate. Parasites were but rarely seen in the proboscis, and then, with one exception, only in small numbers. In the case referred to, trypanosomes were present in the proboscis in considerable numbers, but as both the gut and salivary glands contained enormous numbers of flagellates, their occurrence in the proboscis could easily be explained on the assumption that they had been discharged from the salivary glands and were passing down the proboscis with the secretion of these structures. The parasites from the gut, from the salivary glands, and from the proboscis were inoculated into monkeys and rats, all of which became infected, showing that the trypanosomes in each of these structures were virulent.

As will be seen from Plate XXIII, the parasites present in the gut and in the salivary glands of infective flies exhibit marked differences in morphological characteristics. The forms encountered in the intestine were many and diverse, but the predominant type was undoubtedly that depicted in figs. 7-12. It may be described as a large broad flagellate, with a feebly-developed undulating membrane and little or no free flagellum. The nucleus is usually compact and situated near the centre of the body, but not infrequently it lies in a more posterior position. The blepharoplast is, as a rule, situated near the posterior extremity, but sometimes lies further forward approaching the nucleus. In addition to these forms, long thin parasites were found. Here the nucleus was, as a rule, diffuse, and occupied a central position. The parasites stained more faintly, the undulating membrane was narrow, and there was generally a distinct free flagellum. Many more or less rounded bodies were also seen.

The form met with in the salivary glands of infective flies was almost invariably that represented by figs. 1-5. It approximates somewhat closely to the short form of the trypanosome in the blood of the vertebrate host, but is obviously not identical with this. The nucleus is compact, and is situated at the middle of the body; the blepharoplast is distinct, and lies near the posterior extremity. The undulating membrane is well developed; there is only occasionally a short free flagellum. The length of this form is 15-18μ. In addition to this form, long attenuated flagellates were occasionally seen, but they were very few in number, and it was necessary to search for a considerable period before discovering one of them. These forms resemble the corresponding variety seen in the gut. The nucleus is diffuse, and situated near the centre of the body. The parasite stains a faint pink with Giemsa.

In so far as we could determine, the predominant gut type was not met with in the salivary glands, and the predominant salivary gland type did not occur in the intestine. In spite of this, however, we are faced with the fact that the parasites in both situations proved to be virulent when inoculated into experimental animals. It appears to us that two hypotheses can be advanced to explain this; either that the salivary type is the only virulent form and that a certain number of these reach the intestine from the

salivary glands by way of the proboscis—this may be quite fortuitous, depending on the fact that a certain quantity of infected saliva is from time to time drawn into the intestine—and are then lost amongst the multitude of diverse gut forms, or that there is some unknown form (the one which migrates from the intestine to the salivary glands) which precedes the typical salivary gland type and which is also virulent for laboratory animals.

In conclusion, we might remark that invasion of the salivary glands was only observed in the case of flies infected with the human trypanosome (*T. rhodesiense*) and not in the case of any of the other trypanosomes with which we had to deal either in the Luangwa Valley or on the Congo-Zambesi watershed. This was the case both with the strain of *T. rhodesiense* derived from man and also with that found in 'wild' *Glossina morsitans* which had been infected in nature.

It is of interest to note that of 310 'wild' *Glossina morsitans* which were dissected as they were brought into the laboratory recognisable mammalian red corpuscles were found in the intestine of seventy, whilst nucleated red corpuscles were only found on twelve occasions.

SUMMARY

1. The salivary glands of all *Glossina morsitans* capable of transmitting *T. rhodesiense* are infected, and conversely without invasion of the salivary glands there is no infectivity of the fly.

2. Invasion of the salivary glands is secondary to that of the intestine.

3. The first portion of the developmental cycle of the trypanosome takes place in the gut. In order for its completion and for invasion of the salivary glands to occur, a relatively high mean temperature, 75°-85° F., is necessary.

4. Invasion of the salivary glands was only found in flies infected with the human trypanosome, *T. rhodesiense*.

5. The predominant type of the trypanosome in the intestine of infected *Glossina morsitans*—a large broad form—is quite

different from that which predominates in the salivary glands, where the parasite resembles somewhat the short form seen in the blood of the vertebrate host.

6. Both the intestinal forms and also those from the salivary glands of infective *Glossina morsitans* are virulent when inoculated into healthy animals.

DESCRIPTION OF PLATE XXIII

Trypanosoma rhodesiense in *Glossina morsitans.* Films fixed in alcohol and stained with Giemsa. The figures were drawn with the aid of a camera lucida at a magnification of 2,000 diameters.

Figs. 1-5. Typical forms seen in the salivary glands.

Figs. 6-14. Intestinal forms.

SECTION VI

REPORT OF THE ENTOMOLOGIST,

LLEWELLYN LLOYD

(*a*) *GLOSSINA MORSITANS* IN THE LABORATORY

The breeding of these flies was carried out continuously from the middle of June, 1911, to the end of May, 1912. Kinghorn* has already outlined the method adopted, and has described the larva and pupa. The flies were fed on native fowls at Nawalia, except for a short period when goats were used. At Ngoa the flies were fed at first on fowls, but afterwards entirely upon goats, which died quickly under the repeated feedings, doubtless owing to the trypanosomes with which the flies were naturally infected. Fowls would appear to be unsuitable food, in spite of the fact that the flies feed much more readily upon them than upon mammals. Fowl blood in the 'sucking' stomachs of the flies forms large firm clots, and apparently blood in this condition cannot be utilised by the flies, as the clots have been found to persist for several weeks, monkeys having been used as blood donors in the interim. A fly with so large a clot in the body cannot retain a full-grown larva, and consequently many abort. The phenomenon is rare with mammalian blood, and it has not been observed in nature.

The bottles used for the stock flies were glass cylinders, five inches deep and two inches in diameter. At first only three flies, two females and a male, were kept in each tube, but it was found later that the number could be increased to six without damaging the vitality of the insects. In damp weather many flies die through fouling themselves with the copious semi-fluid faecal deposits. This may be obviated by inverting the tubes over sheets of blotting paper, which absorb the excreta, but precautions must then be taken to avoid the escape of the larvae. This was done by placing the tubes in dishes and paper-lined boxes. When flies are kept under these conditions the daily changing of the tubes becomes unnecessary—a great saving of labour when many flies are kept.

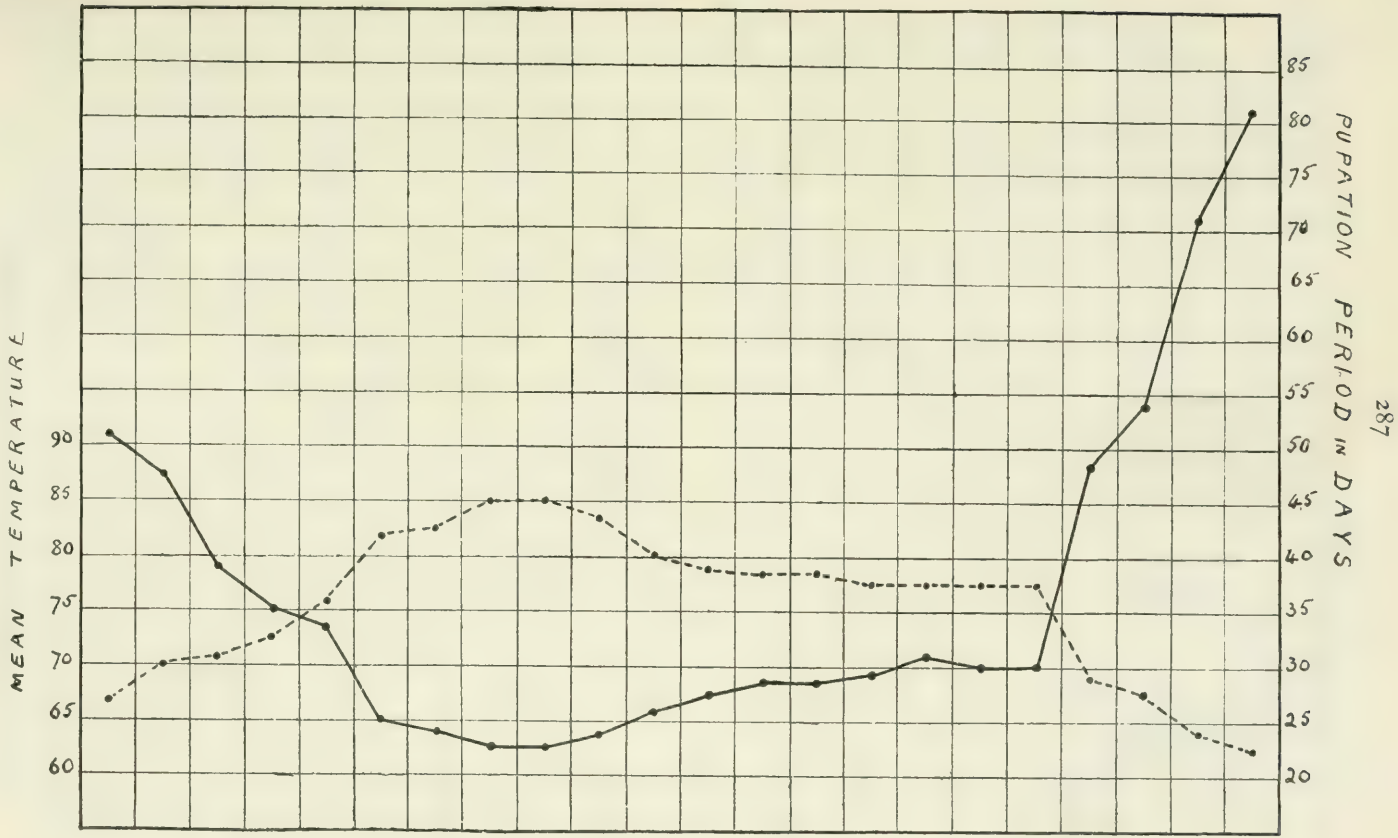
* Kinghorn, A. Bulletin Entomological Research, 1912.

It is sometimes difficult to obtain a sufficient supply of female flies, but natives learn to distinguish between the sexes and to select and bring in females. In a collection made without discrimination, the proportion of females is often as low as 2 %. During the twelve months the number of females in stock has varied considerably. From July to February, with the exception of a period at the end of August when all were destroyed by an accident, the number varied between 200 and 300. During April and May the number of females was between 500 and 600. The flies breed most readily at the beginning and at the end of the rains. This is confirmed by observations on the number of flies in nature at different periods of the year.

Males and females emerged from the pupae in approximately equal proportions for the twelve months. From June to December the males in each month were somewhat in excess of the females, while from January to April the reverse was the case. The pupation period of a male is on the average about a day longer than that of a female.

The variation in the duration of the pupation period is given in Table 47, p. 288. It shows more fully the influence of the climatic conditions on the pupal life; the pupae have been divided into groups which were deposited in periods of half months. For each of these periods the mean temperature in the laboratory and the relative humidity of the air are given. A fuller account of the meteorological conditions is given in a previous section of this report. The approximate mean temperatures to which the various groups of pupae were exposed have been calculated by averaging the mean temperatures for the periods in which the groups were deposited, and for those periods following which could have influenced the duration of the pupation period. Chart 8 indicates how readily the pupae respond to changes of temperature. The humidity of the air has little or no influence on the pupal life, as will be seen by comparing the data of June and July with those of the second half of March. While the relative humidity of the latter portion was 10 % higher than in the former, the temperature and the duration of the pupal life were approximately the same in each case. It should be mentioned that the observations from June to the middle of March were made at Nawalia (altitude 2,100 feet),

CHART 8.—Showing the influence of temperature on the pupation period of *Glossina morsitans*.



The pupation periods are represented by the continuous line, the temperatures by the broken line.

TABLE 47.—Showing the influence of Temperature on the Pupation Period of *Glossina morsitans*.

Period during which the pupae were obtained	Number of apparently healthy pupae	Number of pupae from which flies emerged	Average duration of pupation period—days	Mean laboratory temperature	Approximate mean temperature to which pupae were exposed	Relative humidity of air
June 26—30 ..	3	3	51	—	67° F.	48·6 %
July 1—15 ..	6	5	47	64·1°F.	70	—
,, 16—31 ..	24	18	39	68·3	71	45·7
August 1—15 ..	21	9	35	69·4	73	—
,, 16—31 ..	5	1	33	73·0	76	35·8
September 1—15 ..	2	2	25	72·7	82	—
,, 16—30 ..	14	12	24	80·7	83	31·5
October 1—15 ..	22	15	23	82·9	85	—
,, 16—31 ..	20	10	23	86·4	85	31·8
November 1—15 ..	17	6	24	86·0	83	—
,, 16—30 ...	25	16	26	81·5	80	41·1
December 1—15 ..	27	23	27	80·6	79	—
,, 16—31 ..	13	12	28	78·8	78	69·1
January 1—15 ..	20	16	28	79·0	78	—
,, 16—31 ..	31	28	29	78·0	77	77·7
February 1—15 ..	48	32	31	77·2	77	—
,, 16—29 ..	40	30	30	76·9	77	73·8
March 1—2 ..	11	8	30	78·0	77	—
,, 21—26 ..	4	3	48	75·9	69	62·5
April 1—15 ..	25	16	54	77·2	67	—
,, 16—30 ..	51	22	70	68·0	64	53·7
May 1—15 ..	71	20*	81	70·0	62	—
,, 16—31 ...	96	—	—	63·9	—	51·3
June 1—15 ..	—	—	—	61·8	—	—
,, 16—30 ..	—	—	—	56·9	—	53·0

* On August 4 the remaining pupae were placed in an incubator.

while those for the remainder of the time were made at Ngoa (altitude 4,400 feet).

The shortest pupation period was that of a pupa deposited in the middle of October, from which the fly emerged in twenty-one days. The mean temperature to which this pupa was exposed was slightly over 86° F. The longest period was eighty-eight days for a pupa obtained during May and exposed to a mean temperature of 62° F. Reference to the table will show that these extremely high and low temperatures have a very deleterious effect on the pupae, evinced by the high percentage from which flies do not emerge. Many of the flies which emerged after periods of over seventy days were malformed or weak, and did not survive well. The mean shade temperature of the outside air was always higher than that of the laboratory, and the majority of the pupae found in nature in July were placed in such positions that for several hours a day they would be exposed to the sun, except for a slight covering of soil. It is therefore probable that these very long pupation periods do not occur under natural conditions.

(*b*) A RECORD OF SOME BREEDING PLACES OF *GLOSSINA MORSITANS*

The pupae of *Glossina morsitans* were found in a variety of positions at Ngoa in July; all in the neighbourhood of two permanent streams, the Kanchibia and the Kalamba. At the time, the middle of the dry season, the leaves of the deciduous trees were falling rapidly, and dense shade was only to be found actually in the beds of the streams. The bush is of the nature of an open wood with a large variety of trees. The big trees grow, in general, right to the banks of the streams without intervening plains. Tsetse fly are very numerous in this area.

The following is a list of the positions in which pupae have been found:—

(1) Under a thick branch (native name Mutobo) running about half an inch above the surface of the soil, on gently sloping ground fifty yards from a stream, one living and one empty pupa case were found. They were close to the base of the branch, and were lightly covered with soil.

(2) A similar position under a branch of a tree (native name Muombo) twenty yards from the stream, five cases and numerous fragments were found among dead leaves and humus.

(3) In a hollow in the trunk of a tree (native name Mpasa) a few inches above the surface of the ground, ten yards from the stream. The bottom of the hollow measured about four inches by two, and was full of a firm clay impregnated with resinous matter. Ten pupae, nine cases and numerous fragments were taken from the crevices in the surface, several of them quite exposed.

This spot, visited a few days later, yielded another pupa. The sun shines into this hollow for several hours a day, striking the surface of the soil.

(4) In two large hollows (filled with an old termite nest much broken down) at the base of a tree of the same species on the bank of the stream. One of the hollows was well sheltered and received no sun. In this, fifty cases and numerous fragments were found, but no living pupae. The other hollow received the afternoon sun, and in it one living pupa and several cases were found. (Pl. XXIV, fig. 1).

(5) In a well-sheltered hollow between three main branches of a tree (native name Mupapa), containing dead leaves but no soil or humus, one pupa and eight cases occurred. This tree was on the bank of the stream, and the hollow was about two feet above the ground. (Pl. XXIV, fig. 2.)

(6) In a shallow cup full of soil and roots in a tree (native name Mutobo) close to a stream. This hollow was quite exposed to the sun. One empty case was taken.

(7) One pupa and two cases were found in a hollow full of insect droppings, two feet from the ground, in a tree (native name Muombo). The hollow was exposed to the sun, and the tree was two hundred yards from the stream.

(8) This position resembles the first two. One pupa and one case were found under the trunk of a tree (native name Muanga) which ran for six feet close to the ground before rising. The tree is four hundred yards from water. (Pl. XXV, fig. 3.)

(9) Three empty cases were found in two shallow cups, quite exposed to sun and rain, in a Mutobo tree. The cups contained a little dust and were four feet from the ground, being formed by a swelling on the trunk caused by the pollarding to which the

Awemba tribes subject the trees when making gardens. Three cases were found.

(10) A shallow in a Muombo tree, three feet from the ground, full of loose soil and roots and quite exposed. The tree was two hundred yards from water. Three pupae and five cases were taken.

(11) The stump of a rotten tree built around by termites, which still occupied the base. Twelve living pupae and many fragments were found under the branch and in and about the termite nest. The position was not shaded, and the stump would be well warmed by the sun.

(12) A main branch of a Mutondo tree had been cut across, the cut surface facing upwards. The wood had rotted away internally, forming a cup full of humus and exposed to the sun. Ten living pupae and four cases were found here. (Pl. XXV, fig. 4.)

(13) Two pupae and two cases were found in a hollow in an Mpasa tree two hundred yards from water. The hole was eighteen inches above the ground, and was full of insect droppings.

(14) In the deserted burrow of an ant-bear (*Orycteropus capensis*). The hollow had been occupied and enlarged by warthog during the last rainy season. The burrow ran in such a manner that the morning sun would shine in obliquely or to one side. On this side, and near the opening, nine living pupae and six empty cases were found, but no pupae were found on the sheltered side. In a similar burrow near, which was sheltered from the sun, no pupae were found. This locality was about three hundred yards from water. (Pl. XXVI, fig. 5.)

(15) In a burrow of an ant-bear half a mile from water one empty case was found.

(16) Two living pupae were found in a 'salt-lick' in the side of an ant-hill two hundred yards from water. The position was exposed to the sun. These 'salt-licks' are very common in the district, and are visited by most of the big mammals which scrape and lick away the soil for the sake of the salt it contains until large excavations are formed.

(17) A hollow one foot from the ground in a Musangati tree, full of hard clay and well sheltered, contained two cases.

(18) Ten cases were taken under the trunk of a Mpasa tree

running close to the ground. The position was well sheltered, and was twenty yards away from the water.

(19) A hollow between two main branches of a Chimpampa tree contained two cases. The hollow was filled with dead leaves and humus, and was well sheltered. The tree was close to water.

The pupae were thus found in association with trees of eight different species and in holes in the earth. The native names only of the trees can be given at present. All the trees about which they were found were either abnormal or injured. None have been found at the bases of normal trees or under bushes. The pupae were generally, though not always, hidden under slight covering of earth or dead leaves. These observations confirm those of Jack*, who first recorded the finding of the pupae in nature, that they are deposited in such positions that they are not likely to be scratched up by game birds.

The relation of the positions to shade is of interest, and is probably connected with the season. Of the fifty-four living pupae found, forty-nine (90 %) were so placed that they would be daily warmed by the sun. They were collected in the coldest part of the year, and at a time when flies were emerging from pupae in the laboratory after a period of from seventy-five to eighty-five days. The shade temperatures outside were at the time about 2° F. higher than those of the laboratory, so that in well-shaded positions the pupation period would be about seventy days. This protracted period would be much reduced in pupae which were exposed to the warming influence of the sun.

Reference to Table 47 giving the relation of the pupation period to the mean temperature will show that for temperatures below 70° F., an increase of 1° in the mean temperature causes a reduction in the pupation period of from three to five days. Several of these sunny positions would be waterlogged during rain, and therefore unsuitable for the deposition of pupae.

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* Jack, R. W. Bull. of Ent. Res., Vol. II, pp. 357-361.
Austen, E. E. A Handbook of the Tsetse Flies, London, 1911, p. 56.

(c) A RECORD OF BLOOD-SUCKING INSECTS AND TICKS COLLECTED IN THE LUANGWA VALLEY FROM AUGUST, 1911, TO MARCH, 1912, AND AT NGOA FROM MARCH TO MAY, 1912

Ixodoidea

Ornithodorus moubata, the Nkufu tick, was taken in three villages in the neighbourhood of Chinunda. In two instances it occurred in single huts in the villages in very large numbers, but was said not to have been seen in any other huts. In the third village it was found in two huts, in one of which it was numerous, while in the other, a deserted hut, a single specimen was taken. In this village the information was given that the pest had occurred in almost every hut, but had disappeared from all but the one. The absence of this pest from the Nawalia district is peculiar, as it must be constantly introduced from the higher ground where it is common. The natives attribute the absence to the heat, but a number of Nkufu brought to Nawalia from the above-mentioned villages lived and bred in the laboratory throughout the hot weather.

Tabanidae

These insects, which are very numerous both as regards species and individuals in the Luangwa Valley, were very troublesome in the laboratory, to which they were doubtless attracted by the numbers of animals about the place. Seventeen species of the genus *Tabanus* alone were taken in the laboratory at different times.

Chrysops. Only one species, *C. fuscipennis*, Ric., was seen, and a single specimen was taken at Nawalia in February. It flew out of long grass over swampy ground and settled on the hand. It was so eager to feed that it was readily caught in a tube.

Tabanus

Insects of this genus appeared spasmodically from August to October, the driest months of the year. In November, after the first showers of rain, they become much more numerous, and continued to appear in large numbers throughout the wet months, becoming scarcer again in March. In the latter month several species reappeared which had not been seen for some time, indicating that they were probably emerging from the pupae about this time. Males of several species were also taken in March. The following species were collected:—

T. africanus, Gray. This species was taken at intervals at Nawalia during the rainy season. It was most common during February and March, when it was frequently seen in the laboratory.

T. biguttatus, Wied. Only three specimens of this large species were seen. They were taken in the laboratory and in a canoe in November and February. The males, usually described as being commoner than the females, were not seen.

T. par., Walk. This was one of the most numerous species around Nawalia. It constantly entered the laboratory, and was most persistent in its biting. First seen in October, it became numerous in January, and its numbers declined towards the end of the rains.

T. liventipes, Surc. This somewhat rare species was taken in small numbers at Nawalia in January and February. Specimens were taken on kudu, on roan and about the camp.

T. nigrostriatus, Ric. Hitherto recorded only from Nyasaland and in small numbers, this species was one of the commonest and most troublesome at Nawalia. It entered the laboratory in large numbers, and was often seen on dead game during November and December. It gives very painful bites, settling especially on the head and biting through the hair.

T. taeniola, P. de B. This fly was taken first in October, and became common in November and December at Nawalia. It was again taken in some numbers in March. During the latter month a male was taken at Ngoa.

T. taeniola, var. *variatus*, Wlk. This variety was much more numerous than the typical species. It was first taken in September biting a native at sunset. It was not seen again till November, when it became very troublesome. During January and February it was not observed, but was again taken in some numbers in March.

T. fraternus, Macq. Never very common; specimens of this species were taken at Nawalia in August, November, December and February.

T. fuscipes, Ric. This species first appeared in January at Nawalia, and continued to be numerous to the middle of March. Both sexes were seen in large numbers resting on long grass over a swamp in the evening about the middle of January. In February and March about ninety female specimens of this fly were dissected in connection with the work of the Commission. The gradual maturing of the eggs was thus observed, and early in the latter month some had deposited part of their ova.

T. albipalpus, Wlk. A single female specimen of this species was taken at Ngoa in March. The species has hitherto been regarded as belonging to the West African Fauna, all the specimens having been taken on the West Coast with the exception of one which is recorded from Uganda.

T. pullulus, Acert. This species was taken at Nawalia in small numbers from January to March.

T. claritibialis, Ric. First taken in the laboratory at Nawalia in January. This species was fairly numerous in the two following months.

T. atrimanus, Lw. A single female of this fly was taken in the laboratory at Nawalia during October.

T. copemani, Aust. This species, which has been previously recorded from the Feiera district of Northern Rhodesia and from Nyasaland, was first taken at Nawalia about the middle of January, and in the two following months was one of the commoner species.

T. diversus, Ric. A female specimen of this species was taken in a tent at Kasongo on the Luangwa river in October. One other specimen was found dead on a table in the laboratory, at Nawalia in December. It had been caught by a spider, and was densely wrapped up in a web.

T. maculatissinus, Macq. One female of this was taken feeding on a rhinoceros at Ngoa in May. The distance from water was about a quarter of a mile, and the insect flew to the animal shortly after it had been shot.

In addition to the above, five other species of *Tabanus* were collected at Nawalia and Ngoa. One of these, I am informed, is an undescribed species, specimens of which have previously reached

the British Museum from various localities, and which will shortly be described. The remaining species have not yet been identified.

Haematopota

This genus was not nearly so conspicuous as the preceding one, either as regards species or individuals. One specimen was taken in December, and they were more numerous in January and February. After the latter month they were extremely scarce.

The following species have been identified:—

H. mactans, Aust. A single specimen was taken on a kudu in January at Nawalia.

H. insidiatrix, Aust. This species was several times taken in the laboratory at Nawalia during January and February, and it was also collected on antelope.

H. sp. An undescribed species which has previously been collected in Northern Rhodesia. Several were taken on antelope.

Muscidae. Glossina. The only species of this genus that has been collected is *Glossina morsitans*, Westwood. In the district to which the work of the Commission has been confined this insect is generally distributed. While small areas exist, such as the immediate neighbourhood of Mpika, in which the fly is not actually resident, there is probably no spot in which it does not occasionally occur.

Auchmeromyia luteola, F., is common both in the Luangwa Valley and on the Plateau. During September a large number of huts were searched, and one or more stages of this insect were seen in every hut. The adult insect is to be found throughout the year.

Cordylobia anthropophaga, Grunb. The larone of this fly were several times seen in the legs of wild rats. One larone which was squeezed out of its sac burrowed in sand and pupated, emerging as a fly after a very brief pupation period.

Stomoxys nigra, Macq. A few specimens of this species were collected at Nawalia in January. They were found resting on leaves and grass over a swampy stream.

Stomoxys calcitrans, Linn. (?) was very numerous at Mpika in March about the cattle kraals and on some dogs. In May, when some were required for experimental purposes, only one or two

individuals were found. At this time there had been no rain for two months.

Pupipara

Hippobosca hirsuta, Aust. This species was taken repeatedly on waterbuck (*Cobus ellipsiprymnus* and *C. defassa*), and on puku (*C. vardoni*), both in the Luangwa Valley and at Ngoa. On one occasion a specimen was taken on the back of a native who had been present when a waterbuck was shot a few hours earlier.

Two other species of the *Hippoboscidae* were collected at Nawalia. One of these, a wingless species, was taken on several occasions on bushbuck (*Tragelaphus scriptus*), while the other, a winged species, was only taken on one occasion on hartebeest (*Bubalis lichtensteini*).

My thanks are due to the Entomological Research Committee for the identification of many of the above insects. It would be difficult to exaggerate the usefulness of this institution to Entomologists in the field.

APPENDIX A

AN EXPERIMENT TO ASCERTAIN WHETHER TABANIDS TRANSMIT TRYPANOSOMES IN NATURE

BY

A. F. WALLACE, M.B.

M.O., N. RHODESIA.

AND

LLEWELLYN LLOYD

An experiment to determine whether flies of the genus *Tabanus* were vectors of trypanosomes in nature was carried out at Nawalia. As pressure of work prevented this being done at the time when Tabanids were most numerous, the number of flies used was small and the results are inconclusive. The experiment was carried out during February and March, the last two months of the wet season.

Each morning a number of fly boys were sent out to collect the flies, and brought in daily from ten to thirty, the number being larger when the sun was shining. These were placed in separate tubes, and were fed on a monkey. Only a small proportion of the insects placed on the animal took food. It seemed to make no difference whether the feeding was done in direct sunlight or in the shade.

All the flies that fed were dissected and examined the same day. The dissection of a *Tabanus* is a very simple matter. A needle is thrust through the head and the thorax is held with forceps. The fly is then immersed in salt solution, and a series of gentle pulls is given to the head. The chitin of the neck breaks across, and the alimentary canal is drawn out with the salivary glands. The gut breaks across in the weakest place, just in front of the proventriculus, and the head comes away with the salivary glands attached. To obtain the remainder of the gut in an unbroken condition, the last segment of the abdomen is cut round with

needles, and the gut then drawn out backwards. The proventriculus passes back readily through the thorax and brings with it the trilobed 'sucking stomach.' Microscope preparations were then made of the proboscis with the pharynx, the complete gut from proventriculus to anus, and the salivary glands. These preparations were examined for parasites.

In all, 128 flies were fed and dissected. These comprised the following species:—

T. fuscipes, Ric.	... ...	87 flies.
T. taeniola, P. de B.	... ...	25 ,,
T. claritibialis, Ric.	... ...	2 ,,
T. copemani, Aust.	... ...	10 ,,
T. pullulus, Aust.	... ...	2 ,,
T. par, Wlk.	... ...	2 ,,

These flies were fed between February 7th and March 15th. The monkey did not become infected.

Flagellated parasites were found in seven flies. In no cases were they seen in the proboscis or the salivary glands, but were confined to the mid- and hind-gut. Smears were made of the preparations, and in four instances inoculations were made into wild rats. Only two of the rats lived long enough for any conclusions to be drawn, and these remained negative.

APPENDIX B

AN ATTEMPT TO TRANSMIT *TRYPANOSOMA RHODESIENSE* BY MEANS OF *ORNITHODOROS MOUBATA*

BY

A. F. WALLACE

An attempt was made at Nawalia to transmit *T. rhodesiense* by *Ornithodoros moubata*. For this purpose ticks had to be brought down to Nawalia from the plateau at Mpika. It is a curious fact that there are no 'nkufu' (the native name for these ticks) in the Luangwa Valley, but that they abound in the hilly country on either side. This fact is well recognised by the natives, who explain it by the Luangwa Valley being too hot for ticks to live in. This may be the explanation of the high death rate in the experimental ticks.

Five experiments were conducted, about twenty-five ticks being used in each. Each group of ticks was fed on a monkey heavily infected with *T. rhodesiense*, and after an interval of about a month was fed on a clean monkey. Each lot of ticks was kept under observation for six to seven months, being fed at intervals on clean monkeys. Only a few of the ticks survived for seven months.

The following is a summary of the experiments:—

To begin with, 125 ticks were fed on a heavily-infected monkey.

After one month 87 ticks were fed on a clean monkey.

,, two months 51 ,, ,, ,, ,, ,,

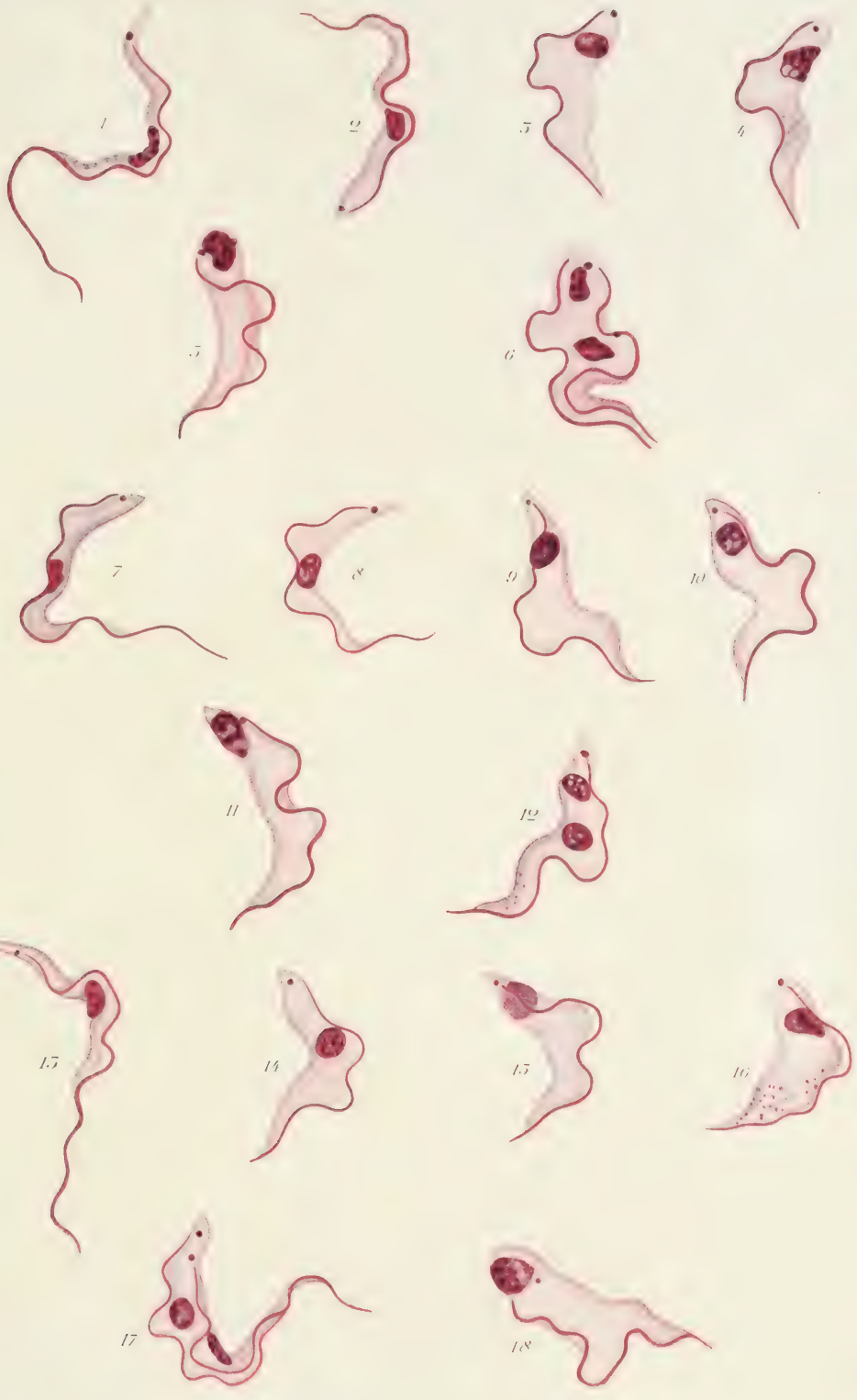
,, three ,, 8 ,, ,, ,, ,, ,,

,, six ,, 23 ,, ,, ,, ,, ,,

,, seven ,, 10 ,, ,, ,, ,, ,,

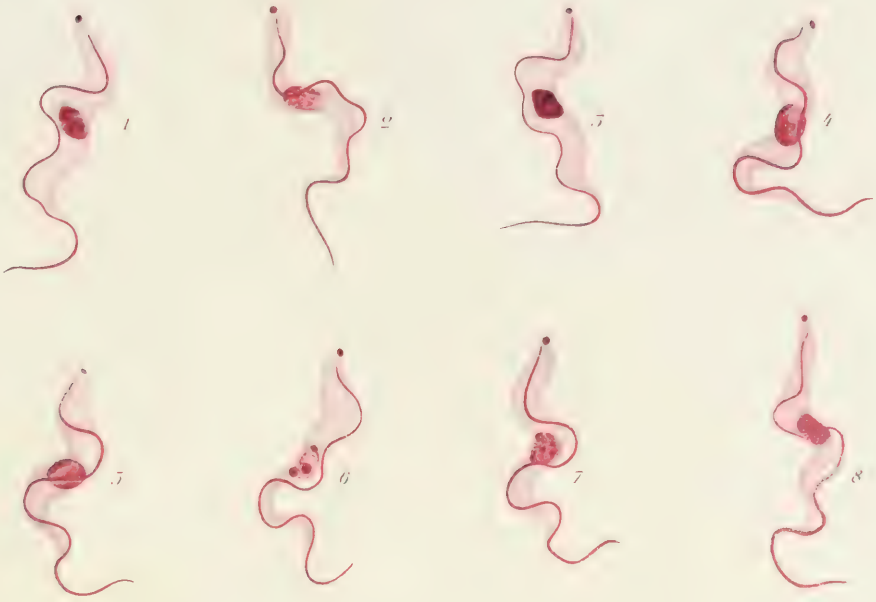
The blood of the clean animals remained negative.

The result of interrupted feeding was also investigated. After the ticks had become partially distended with blood they were removed from the infected monkey and transferred to healthy animals. Certain of the ticks would not continue their meal, and in the case of those which did there was always a short interval, varying from a half to five minutes before they resumed. In all, sixty-one ticks were used in these experiments to ascertain whether the trypanosome could be transmitted from one infected animal to a healthy one by means of interrupted feeding. The result was negative in every instance.

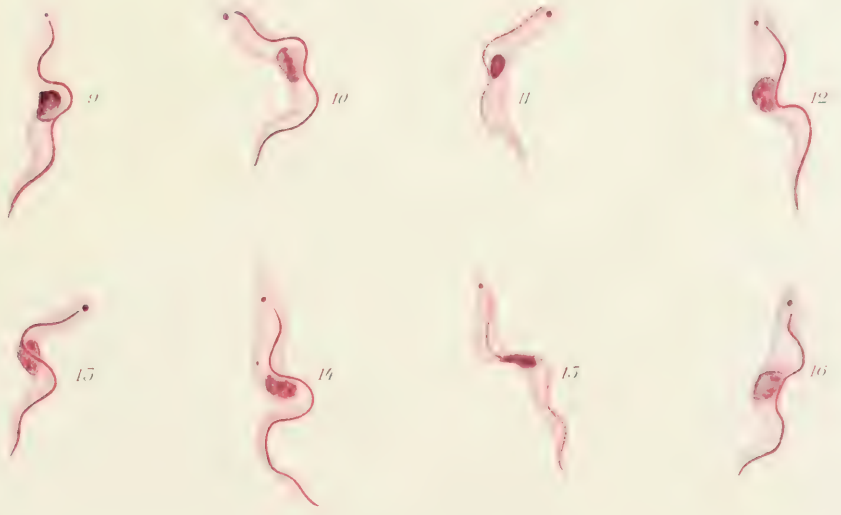


TRYPANOSOMA RHODESIENSE

A. M. Brookfield, del.

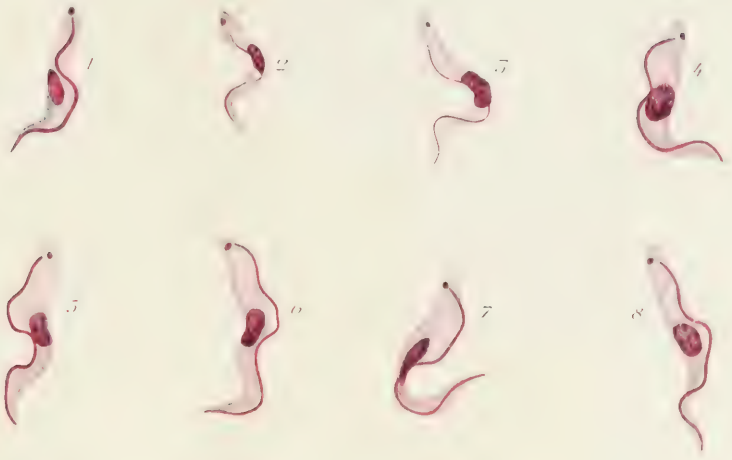


TRYPANOSOMA VIVAX

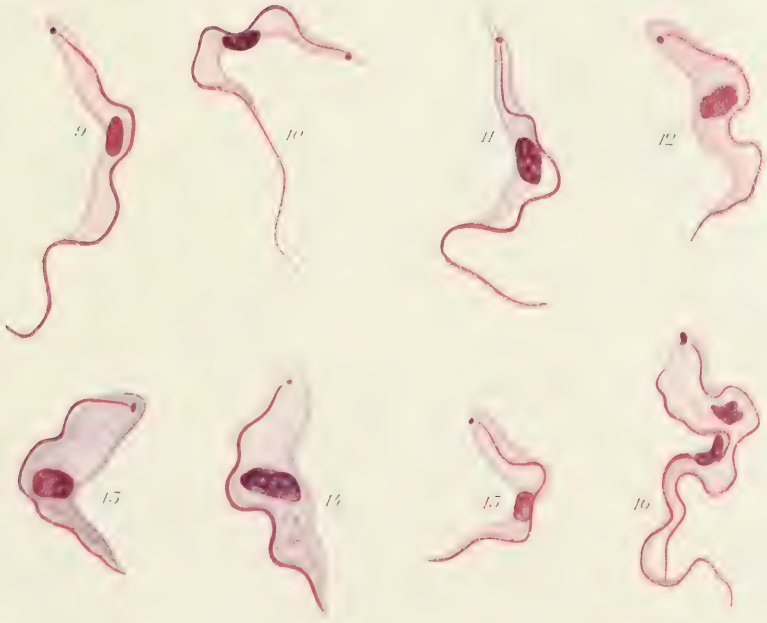


A. M. Brookfield, del

TRYPANOSOMA NANUM

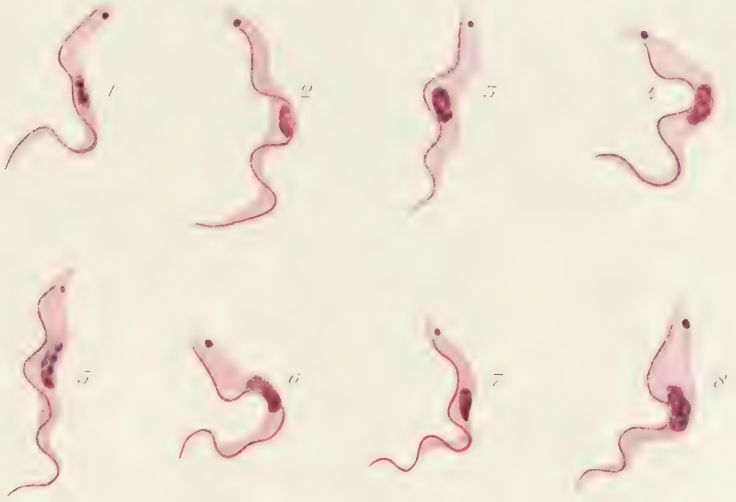


TRYPANOSOMA PECORUM

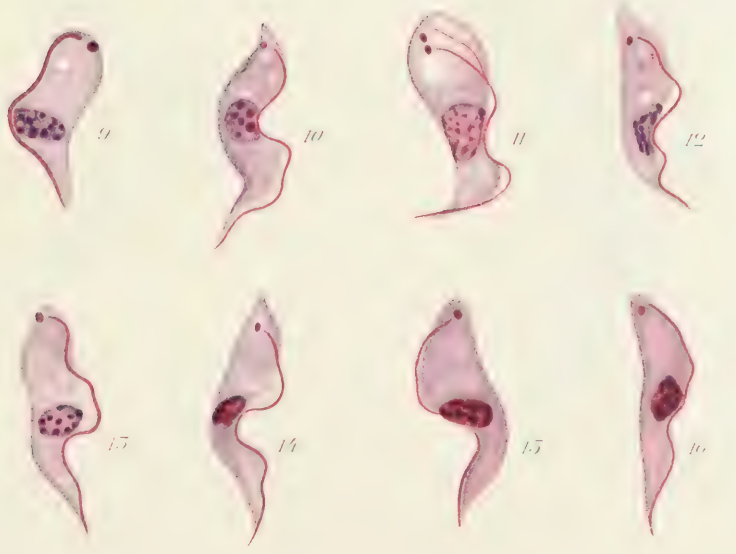


TRYPANOSOMA MULTIFORME

A. M. Brookfield, del

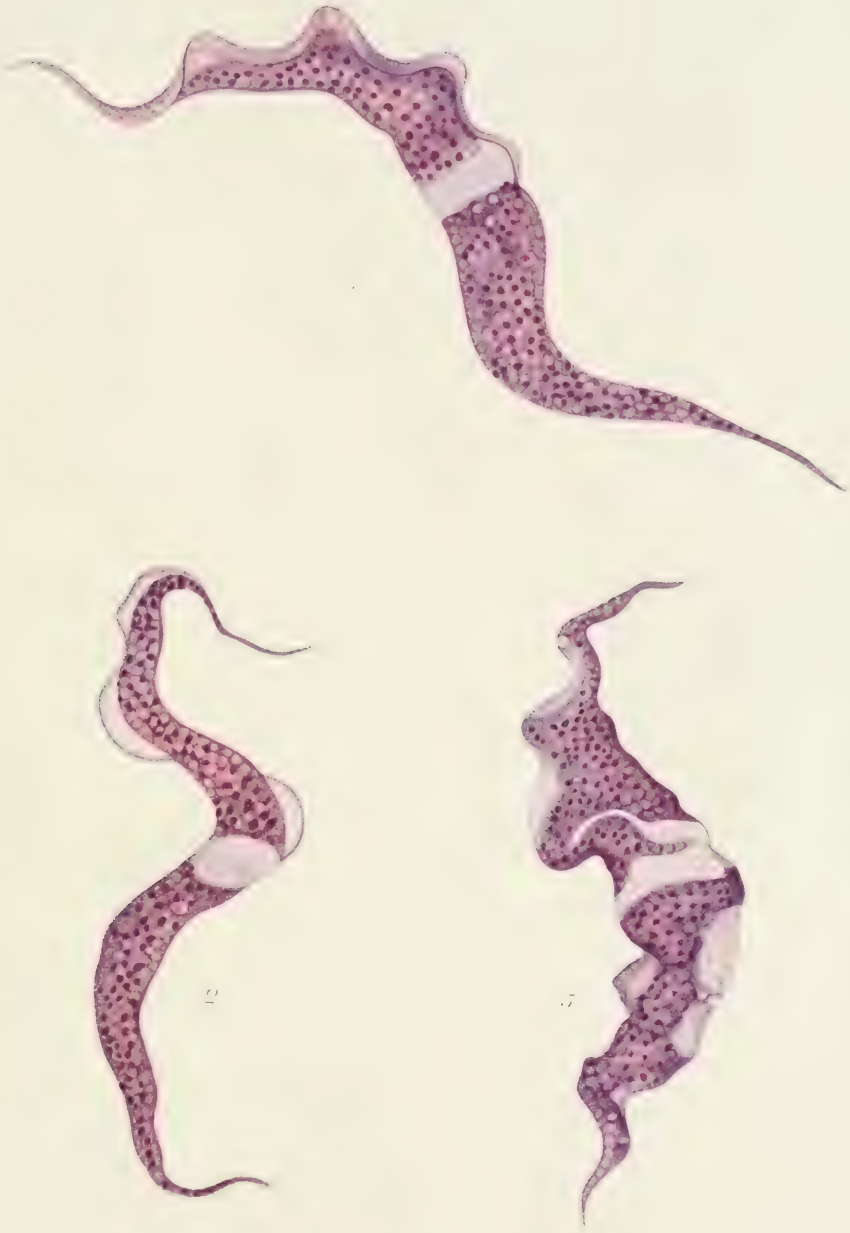


TRYPANOSOMA IGNOTUM



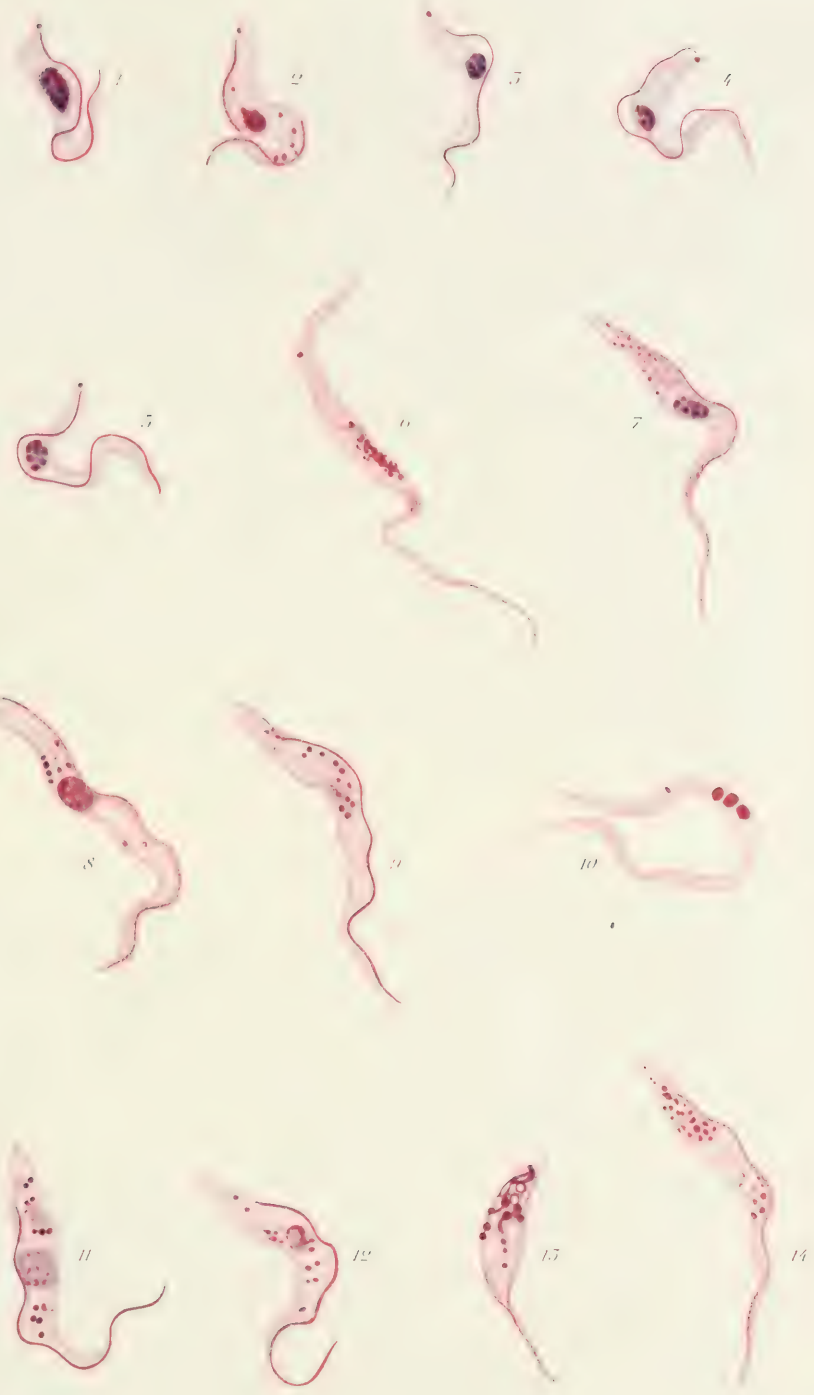
TRYPANOSOMA MONTGOMERYI

A. M. Brookfield, del



TRYPANOSOMA TRAGELAPHI

A M Brookfield, del



A. M. Brookfield, del.

TRYPANOSOMA RHODESIENSE
IN GLOSSINA MORSITANS

FIG. 1. Pupal habitats of *Glossina morsitans.*

FIG. 2. Pupal habitats of *Glossina morsitans.*

C. Tinling & Co., Ltd., Imp.

FIG. 3. Pupal habitats of *Glossina morsitans*.

FIG. 4. Pupal habitats of *Glossina morsitans*.

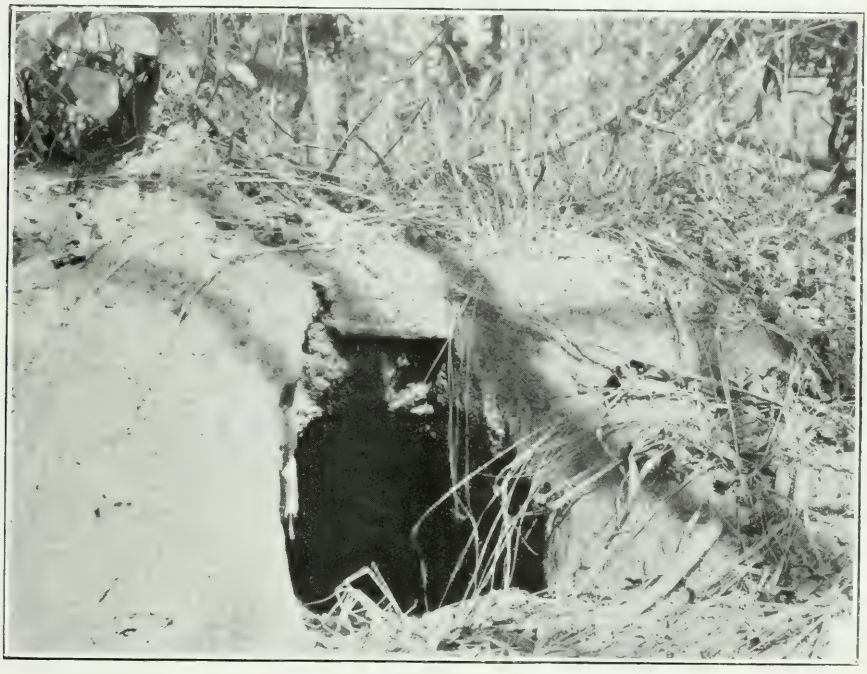


FIG. 5. Pupal habitats of *Glossina morsitans.*

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